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ERRATA

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Page 344, line 18: *for* 'Granlund' *read* 'Gross'

Also on page 346, line 6: delete one 'also'

THE NEW PHYTOLOGIST

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A CRITICAL REVISION OF CERTAIN TAXONOMIC GROUPS OF THE MALVALES

By H. L. EDLIN, B.Sc.

INTRODUCTION

THE Malvales constitute a natural order of plants of the first importance both from a botanical and economic standpoint. They form the sixth cohort of Bentham and Hooker's Polypetalae, and the twenty-sixth order of Engler and Prantl's Archichlamydeae.

The best known members of the order are *Malva*, the mallow; *Althaea*, the hollyhock; *Adansonia*, the baobab; and *Tilia*, the linden tree. The following useful products are obtained from this order: cotton, from *Gossypium* species; jute, from *Corchorus olitorius*; kapok, from *Ceiba pentandra*; silk cotton, from *Bombax malabaricum*; cocoa, from *Theobroma cacao*; and many others of minor or purely local importance. *Heritiera*, *Tarrietia*, *Cistanthera*, *Mansonia*, *Tilia*, *Bombax*, *Ochroma*, and others, yield valuable timbers, some of which are among the lightest in weight known. Several genera furnish shade trees and flowering plants of great horticultural value in both tropic and temperate zones. *Gossypium* and *Theobroma* yield official substances.

The order comprises some 223 genera and 2380 species, which are distributed throughout the tropical and temperate regions of both hemispheres. Four genera, *Althaea*, *Lavatera*, *Malva*, and *Tilia*, occur wild in Britain. The Malvales are arborescent in the main, but vary from forest trees of the first magnitude (*Sterculia*) to cushion plants only a few inches high (*Nototriche*); none are aquatic, though many are coastal halophytes (*Heritiera*, *Hibiscus*, *Thespesia*, *Althaea*, *Lavatera*). They are found in the tropical rain forest and on the heights of the Andes, but are absent from high latitudes. Only *Nototriche* is markedly xerophytic in structure.

Characteristic of the order are the stellate hairs, which are replaced in certain genera by scales; neither of these structures appears to be of special functional importance. No plants of the order

are poisonous. The majority have tenacious fibrous inner bark or bast, and mucilage canals, especially in the unligified organs; mucilage is lacking from the section *Elaeocarpaceae* of some writers, but it is often replaced by a bitter principle.

It is remarkable that in so well-known and widespread an order, no general agreement as regards subclassification has been reached. This is doubtless attributable to the peculiarity and homogeneity of the Malvales. Superficially, they all appear very similar, and only a careful examination can reveal the underlying differences which break them up into distinct and definite families.

From the point of view of practical forestry, it is of importance to know just how far these families extend. The object of the present study is to define the families of the Malvales by characters which are readily ascertainable, and to revise the more detailed classification of certain families. The recent publication of two papers(4, 5) on the microscopical wood structure of the order, has afforded valuable data not hitherto available.

FORMER CLASSIFICATION

Linnaeus(12) in *Philosophia Botanica*, 1763, was the first to apply the name "*Columniferae*" to one of his natural groups or "fragmenta." This included the genus *Gossypium* and most of the modern *Malvaceae*; *Theobroma* was placed with the *Tiliaceae* in the group *Culminiae*; but both these groups included genera now excluded from the Malvales.

The *Genera Plantarum* of Bentham and Hooker(2) gives the first sound and practical classification of the group. In this, the small family *Chlaenaceae* is placed in cohort V, *Guttiferales*, which have stamens indefinite and calyx imbricate; and cohort VI, *Malvales*, is defined, within the series *Thalamiflorae* of the *Polypetalae*, as having the stamens indefinite and the calyx valvate. The placentation is defined as axile.

The cohort *Malvales* is divided into three families, the *Malvaceae*, which include the *Bombaceae*, are distinguished by their unilocular anthers, and the *Tiliaceae* by their free stamens. No precise distinction is drawn between the *Tiliaceae* and the *Sterculiaceae*, which are described as having stamens monadelphous, or opposite the petals in clusters or singly; this description would apply very well to many of the *Tiliaceae* (e.g. *Tilia*).

Nevertheless, this classification of the families would be satisfactory but for the fact that the families are too large and hetero-

geneous for practical use in determining a specimen. Later writers have endeavoured to remedy this by splitting off certain tribes as families.

This arrangement is adhered to by K. Schumann in Engler and Prantl's *Die Pflanzenfamilien*(7), except that the Bombaceae are raised to the status of a family, and the tribes Sloaniaceae and Elaeocarpeae are put apart from the rest of the Tiliaceae in a new family Elaeocarpaceae. But in both cases no precise dividing line is given for the new families, and the rearrangement has little to recommend it.

Warming's(16) arrangement of the group Columniferae is of interest, because he attempts to set the families in evolutionary sequence. Adopting the viewpoint that flowers with few floral parts are primitive, he calls upon the theory of chorisis to explain the evolution of the group Malvales. He says of the family Sterculiaceae (including Buettneriaceae): "This is, no doubt, the least modified order, and one in which the stamens occur undivided"; and of the Tiliaceae: "This differs from the other orders of the Columniferae chiefly in the stamens being entirely free from each other, and, also divided into many filaments, as far as the base, or at all events very far down, so that the flower appears to have numerous stamens, or to be slightly polyadelphous." The third family, Malvaceae, has: "5 apparently numerous stamens, with the filaments united into a tube, with reniform unilocular anthers." The significance of the phrase "5 apparently numerous" is obscure; if a *Gossypium* may be said to have five stamens, so may a *Magnolia*; the description may fit the theory, but it does not fit the plants.

Speaking of the Malvaceae, which include the Bombaceae, Warming says: "The order is the most advanced type of Columniferae; it stands especially near to the Sterculiaceae, but is separated from these, and from the Tiliaceae, by its unilocular anthers." The subdivision within this family is noteworthy; Group 1 has the "carpels in one whorl"; Group 2 has the "carpels arranged in a spherical head in five groups opposite to the petals," and contains only the Malopeae. Group 1 is further divided into Section A, "The fruit is a capsule, most frequently with loculicidal dehiscence, and many seeds in each locus"; and Section B, "Schizocarps, with 1-seeded fruitlets, most frequently nut-like and reniform." Section A consists of the Gossypieae (including *Hibiscus*), and the Bombaceae, which last are distinguished by a divided staminal tube and a simple style. Section B includes the Malvaceae and the Urenaee, the latter "having twice as many styler branches as carpels."

The recognition of an affinity between the Gossypieae (Hibisceae of other authors) and the Bombaceae is of importance here.

Hutchinson (9) takes the bold step of setting apart the Malvaceae (*excluding* Bombaceae) in an order of their own, the "Malvales." He regards them as "A very natural group representing a fixed type of the Tiliales" from which order they are separated as being "herbaceous or softly woody," whereas the Tiliales are "trees or shrubs." This is no doubt true in the main, but it is stretching a point very far to describe certain species of *Corchorus* (Tiliaceae) as shrubs, or *Thespesia* (Malvaceae) as softly woody; and numerous other examples might be cited. It is interesting to note that Theophrastus (*circa* 300 B.C.) encountered the same difficulty, for he was uncertain as to whether mallow should be classed as a herb or a tree.

In criticism of Hutchinson's arrangement, it must be said that he rejects an obvious and clear-cut character, the unilocular anther, in favour of a vague and variable one, the habit of the plants. Thereby he obscures the affinity between the Bombaceae and the Malvaceae proper, forms a rather heterogeneous group of plants in his Tiliales, and creates an unmerited gap by placing the Malvaceae in another order.

In the less detailed treatises, the Malvales receive a rather inadequate and uneven treatment; but this is no doubt due to the fact that only *Tilia* (Tiliaceae) and certain genera of the Malvaceae, are common in the north temperate region. Some writers take *Sterculia* as being a typical genus of the family Sterculiaceae, but actually this genus and the other Sterculieae are marked exceptions to the general type.

THE STERCULIEAE

So far as the author is aware, no writer on the floristic taxonomy of the Malvales has suggested that this tribe should stand apart as a distinct family. This is remarkable, since it is almost the only tribe as to the composition of which all the authorities are agreed. Alphonse De Candolle (*vide* Lindley (11)) suggested that it should be set apart from the Buettnerieae and their allies in his "Sterculiaceae," which also included the Helicterae and the Bombaceae. But there are abundant grounds for the promotion of the single tribe to the status of a family, and these will be considered in detail.

The Sterculieae are the only Malvales in which the flowers are both apetalous and unisexual, or at least unisexual in function; combined with this is an apocarpous fruit and an invariably arborescent habit. The number of genera varies largely, owing to the varying

degrees of subdivision of *Sterculia* according to different writers; but the chief genera are: *Sterculia*, *Tarrietia*, *Octolobus*, *Basiloxylon*, *Cola*, *Heritiera* and *Tetradia*. The distribution is predominantly palaeotropical, the bulk of species occurring in Indo-Malaya, Australia, and tropical Africa, with a few outliers (*Basiloxylon* and *Sterculia* sp.) in western South America; but this extension of distribution across the Equatorial Atlantic is found in many families (cf. Vochysiaceae), and, though it may eventually throw some light upon its phylogeny, is not peculiar to this tribe.

The occurrence of either apetalogy or unisexuality is by no means unusual in the Malvales, and appears to have little systematic significance; but the association of these characters is only found within the Sterculiaceae, and suggests a definite trend towards a general reduction of floral structure such as is found in Bentham and Hooker's (2) series Unisexuales of their Monochlamydeae.

The Sterculiaceae are atypical when considered as members of the Sterculiaceae of Bentham and Hooker (2), but no mention of this is made by either Engler and Prantl (7), or Hutchinson (9), who adopt that family without substantial alteration. Typically its members show a reduction in the androecium, either to staminodes alternating with fertile stamens, or to five stamens (e.g. *Dombeya*, *Hermannia*, *Buckneria*); but the Sterculiaceae never have staminodes, and their stamens only number less than ten in exceptional cases (e.g. four in *Tetradia*). They are more arborescent in habit than the typical Sterculiaceae, in which group complete apetalogy is rare, and unisexuality and apocarpus are unknown.

The separation of the Sterculiaceae as a distinct family would greatly simplify the subclassification of the order Malvales. A definition which is broad enough to include them within the existing Sterculiaceae, besides being cumbersome to apply, is also apt to include a few of the less typical Tiliaceae and Bombacaceae. But if they are defined as a separate entity, much of this confusion disappears; and the keying out of specimens is also simplified.

Although the Sterculiaceae show no deep affinities with the Bombacaceae or with other Sterculiaceae, they may easily be linked up with the Tiliaceae, probably the most primitive family of the Malvales, through the genus *Christiana*. It is significant that the range of this genus extends into both the Old and New Worlds, and into regions where the Sterculiaceae occur; this may account for their distribution into America.

Bentham and Hooker's (2) choice of the title "Sterculiaceae" for

their reconstituted family was an unfortunate one, since later writers, who have not made a special study of the group, have assumed *Sterculia* to be the typical genus, and have gained a misconception of the whole family; and to this the general obscurity which surrounds it is largely due.

The separation of this tribe would in no way lessen the general standing of the families as units; for many families, and even orders, are generally recognised which are not so sharply and obviously distinguished from their congeners (cf. Hutchinson's "Key to the Families of the Dicotyledons" (9), for examples) as are the Sterculiaceae.

After a careful examination of the general anatomy of all the Malvales of which he had material, Dumont (10) sums up the Sterculiaceae by citing five distinct anatomical features, which "make the Sterculiaceae a group parallel to the primary families Malvaceae, Bombacaceae, and Tiliaceae."

Following an investigation embracing the wood of sixty-five species of the Sterculiaceae, a total of twenty-three genera of the Sterculiaceae, and numerous examples of other families of the Malvales, Miss Chattaway has made the following statement: "The Sterculiaceae are all very unlike the rest of the family. Should this subgroup stand apart as a different family?" The chief distinguishing features of the group are the absence of the cells from the rays, the presence of sheath cells surrounding the rays (found in only one genus outside the group), and the occurrence of broad bands of parenchyma in at least some species of each genus. (This last feature is not found in any extraneous genus, but is absent also from *Heritiera*.) No other tribe of the Sterculiaceae is isolated as regards its wood structure.

The only argument for retaining the Sterculiaceae within the family seems to be their supposed affinity with the Helicteraceae and *Eriolaena*, which were included in De Candolle's original Sterculiaceae (*vide* Lindley (11)). But members of the Helicteraceae have always a well-developed androgynophore, and both they and *Eriolaena* have a petaloid, bisexual flower and a syncarpous fruit; staminodes also occur. There is no special affinity in the general anatomy of the two tribes and their wood is very different.

The separation of the Sterculiaceae is desirable on account of the structure of their wood, vegetative organs, flowers, and fruit, and because it renders the subdivision of the order Malvales simpler to apply and easier to understand. It is therefore suggested that the family "Sterculiaceae" should include the Sterculiaceae and the Sterculiaceae only.

The name "Buettneriaceae" first used (with the spelling "Byttneriaceae") by Alphonse De Candolle in his *Théorie Élémentaire de la Botanique*, published in 1819 (*vide* Lindley(11)), is suggested for the remainder of the family. This will include all the Buettneriaceae of De Candolle and of Lindley, comprising the five tribes: Lasiopetaleae, Buettneriaceae, Hermannieae, Dombeyae, and Eriolaeneae, together with the Helictereae (transferred from their Sterculiaceae), and the Mansonieae (only recently discovered).

SUBDIVISION OF THE STERCULIEAE

The original subdivision of this tribe, proposed by Bentham and Hooker(2) and generally adopted since, runs as follows:

- A. "Antherae inordinate congestae. Semina albuminosa." Genera: *Sterculia*, *Tarrietia*, *Octolobus*.
- B. "Antherae uniseriatim annulatae. Albumen O." Genera: *Cola*, *Heritiera*, *Tetradia*.

For the following reasons this distinction has proved impracticable: The presence or absence of albumen, taken alone, is hardly a sufficient basis for the recognition of *species* as distinct. Further, its detection requires careful dissection, and the fruit must be available; hence it is an unsuitable criterion in this group, as the fruit takes a long time to mature. The congested anthers can only be seen in the male flower; in the female flower the infertile anthers are *always* in a ring around the carpels; and even in the male flower it is often difficult to decide whether they are congested or ranged in a ring, especially where (as in *Cola acuminata*) the ring has two tiers of anthers.

Bentham and Hooker's distinction has proved confusing in the following cases: *Sterculia foetida* L., a widespread species, was introduced into Nigeria, where it was later identified as a species of *Cola*, although the genus *Sterculia* occurs there. This was due to the anthers of the male flower being often ranged in a ring; one flower examined had ten anthers inserted on short filaments in a symmetrical circle. The genus *Pterocymbium*, which in Engler and Prantl(7) appears under group B, was previously a part of *Sterculia*, and so in group A; on the other hand, *Pterygota*, which is also derived from *Sterculia*, and is retained by Engler and Prantl in group A, has (at least in the species *P. macrocarpa* K. Schum.) its anthers beautifully ranged in a ring, and so comes into group B. Hutchinson and Dalziel's key in

their *Flora of West Tropical Africa*(10) shows this, and also the ambiguous position of *Tarrietia*. It runs:

"Anthers arranged in an irregular mass—*Sterculia*, *Firmiana*.
Anthers arranged in whorls—*Cola*, *Pterygota*, *Tarrietia*."

Tarrietia was the subject of a special note by Sprague in the *Kew Bulletin* for 1916(15). He named the only West African species, *T. utilis*, after he had discovered that the seed was albuminous. It had previously been ranked as a *Heritiera*, since the anthers of the male flower are ranged in a beautiful ring. He states: "The character of the annular or irregular arrangement of the anthers is very misleading, as there is great variation in this respect within the limits of a single genus, certain species of *Sterculia* (e.g. *S. foetida*) and *Tarrietia* having the anthers distinctly arranged in a ring, so that, as far as the flowers are concerned, they might be taken for species of *Cola* and *Heritiera* respectively. A more natural grouping would be to associate on the one hand *Sterculia* and *Cola*, which have pluri-ovulate carpels and follicular fruit, and on the other hand *Tarrietia* and *Heritiera*, which have uni-ovulate carpels and indehiscent sycamore-like samaras."

Octolobus is another genus which has been placed by Bentham and Hooker in group A, but the specimens examined have the anthers arranged in the simple ring characteristic of group B. Schumann, in Engler and Prantl(7), retains it in group A, but in Engler's *Sterculiaceae Africanæ* (14) he says of the anthers of *O. spectabilis* "thecis c. 50 uniseriatim dispositis."

The subdivision of the Sterculiaceae here proposed is on the lines suggested by Sprague and is briefly this:

I. Indumentum stellate, ovules and seeds numerous, carpels wingless, dehiscent.

Tribe: Sterculineae. Genera: *Sterculia*, *Cola*, *Octolobus*, *Tetradia*.

II. Indumentum lepidote, ovules 1-2 per loculus, seed solitary, carpels with a large or rudimentary wing, indehiscent.

Tribe: Tarrietieae. Genera: *Tarrietia*, *Heritiera*, *Argyrodendron*.

This subdivision has the advantage of being applicable to the leaves or to the female flowers or fruits without the leaves. It is backed by other evidence. Miss Chattaway(4) found that the wood of *Tarrietia* and *Heritiera* was different in many respects from that of other genera of the Sterculiaceae, though the two genera agree with one another, and she goes so far as to suggest that they should be excluded from the subfamily. Dumont(6) found that *Tarrietia argyro-*

dendron Benth. closely resembled *Heritiera* in its anatomy, and that the genus *Cola*, except for two points of minor significance, came very close indeed to the genus *Sterculia*.

THE TRIBE STERCULINEAE

Two genera of this tribe may be easily distinguished; *Tetradia* has its flower parts in fours, and *Octolobus* has an eight-lobed calyx and very numerous free carpels. The remainder are not so readily distinguishable; the *Colas*, which are confined to Africa, have their seed exalbuminous, and their anthers in a more definite ring, but are not sharply separated from *Sterculia*. The other genera have all at one time or another been included in *Sterculia*; the generic distinctions are mainly based on the fruit; until this group, numbering at least 150 species scattered throughout tropical Asia, Africa, Australia, and South America, has been revised monographically by one investigator, it will be impossible to say with any finality what genera should or should not stand. *Cola* must be regarded as a subgenus of *Sterculia*, other subgenera of the same standing being: *Brachychilon* Endl., *Pterygota* Endl., *Firmiana* Marsigli, *Pterocymbium* R. Br., *Basiloxylon* K. Schum., *Eriobroma* Pierre, *Scaphium* Endl., *Erythropsis* Lindl., *Carpophyllum* Miq., and *Hildegardia* Schott.

It is possible that a careful consideration of the wood structure of the Sterculineae may throw light on the value of the generic divisions.

THE TRIBE TARRIETIEAE

When the fruits of this tribe are examined, the species will be found to be divisible into the following three genera: wing of carpel rudimentary, *Heritiera*, wing developed, constricted at base, *Argyrodendron*; wing developed, not constricted, *Tarrietia*.

Heritiera Ait. has four species scattered along the coasts of the tropical Old World, with woody carpels, well adapted to marine distribution; the wood of *H. fomes* is valued for fuel in the neighbourhood of Calcutta.

Tarrietia Bl. has about ten species in Indo-Malaya, and one (*T. utilis*) in West Africa; some species yield useful timber. In the eight species the fruits of which I have examined, there is no sharp line of division between the swollen part of the carpel and the wing.

In *Argyrodendron*, which has two species in Australia, there is a distinct constriction separating the wing from the seed; this suggests a line of evolution from *Tarrietia* towards more effective wind

distribution; the wing is far larger in proportion to the seed than is the case in *Tarrietia*. The wood structure of *Argyrodendron* is of a type different from that of either *Tarrietia* or *Heritiera*.

The name "*Argyrodendron*" was first published by F. von Mueller in his description of *A. trifoliolatum* in his *Fragmenta*, 1, 2 (1858). Benthams and Hooker(2), and Benthams in the *Flora Australiensis*, referred that species to *Tarrietia* under the name *T. Argyrodendron*; at this date, however, Benthams was familiar with only one other species of *Tarrietia*. But if the genus is kept distinct, the original name, *Argyrodendron trifoliolatum* F. von Mueller, must stand. The other species was first described by C. Moore (in 1893), and was described (in 1899) by F. M. Bailey (*vide* Maiden(13)), again under the name of *Tarrietia actinophylla*; the name *Argyrodendron actinophyllum* (Moore) Edlin comb.nov. is therefore proposed for it. A third species, described by von Mueller in *Fragmenta*, 9, 43, under the name of *Tarrietia trifoliolata* does not appear to be distinct from *Argyrodendron trifoliolatum*.

THE MANSONIEAE

Cistanthera K. Schum. consists of some four species of valuable West African timber trees. *C. papaverifera*, which provides the wood known there as Danta or Otutu, is remarkable in that its woody fruits closely resemble the capsules of species of *Papaver*.

In the 1897 "Nachträge" to Engler and Prantl(7), the genus is referred, presumably by Schumann himself, to the tribe Tiliace of the Tiliaceae. Within that tribe it keys out, on account of the presence of staminodes, in the same group as *Corchoropsis*; it is perhaps significant that the latter genus has since been transferred to the Sterculiaceae. Burret excludes *Cistanthera* from the Tiliaceae, but his proposal to transfer it to the tribe Dombeyace of the Sterculiaceae seems very wide of the mark; Dombeyace are essentially characterised by a staminal column, but no trace of that structure appears in *Cistanthera*.

In 1905, Prain (*vide* Engler and Prantl(7)) published a description of "a new tribe of the natural order Sterculiaceae," which he called the Mansonieae, in which he placed the two genera *Mansonia* and *Triplochiton*. *Cistanthera* agrees with the description of that tribe, as published in the third "Nachträge" to Engler and Prantl, in every particular, except that it lacks an androgynophore. But it seems possible that a conspicuous swelling on the pedicel of *Cistanthera* represents the base of an androgynophore which has become obsolete owing to the adnation to it of calyx and corolla; in any case it is significant that

a precisely similar swelling occurs on the pedicel of *Triplochiton*, though in this case it is relatively nearer to the insertion of the sepals. Other points of resemblance between the three genera are their habitat, since species of all three occur in the forests of West Africa, where they form large timber trees; and their wood, that of *Mansonia* approaching that of *Cistanthera* so closely in general appearance, that the native name "Otutu" is applied indiscriminately to either; the woods of both these species possess prominent ripple marks, which are an unusual feature; whilst their microscopic structure is similar.

It is doubtful whether the absence of an androgynophore is sufficient to exclude the genus *Cistanthera* from the Mansonieae, especially as the androgynophore scarcely exceeds the ovary in length, and is never a prominent feature. The fruit of *Cistanthera* differs markedly from that of the others, as the seed, and not the carpel, is winged. But in the allied Sterculiaceae, this difference is found not merely within a tribe, but within a genus; for some writers include *Scaphium* Endl. with winged carpels, and *Pterygota* with winged seeds, in the same genus *Sterculia*. The ovary of *Cistanthera* resembles that of *Mansonia* more closely than does that of *Triplochiton*; for in the former cases the numerous ovules are in two ranks in each carpel; while in the last-named there is only a single ovule in each of the five carpels.

It is therefore suggested that *Cistanthera* be transferred from Tiliaceae to the tribe Mansonieae, which it is proposed to include in the revised family Buettneriaceae. The distinguishing features of this tribe may be defined as follows:

Trees. Anthers ten to many, free or very shortly united, in groups alternating with five petaloid interior staminodes.

The tribe Mansonieae may be split up into its three constituent genera in the following way:

- A. Calyx campanulate; stamens 20-30. *Triplochiton*.
- B. Sepals free; stamens 15. *Cistanthera*.
- C. Calyx spathaceous; stamens 10. *Mansonia*.

THE ERIOLAENEAE

Eriolaena DC. is the only genus of the tribe Eriolaeneae, both of Bentham and Hooker(2), and of Engler and Prantl(7), by whom it is placed in the Sterculiaceae. The eight species are all trees, confined to the Indo-Malayan region.

Eriolaena approaches very closely to the genus *Pterospermum* of

the Helictereae in the following particulars: the flower is subtended by three characteristic lacinate fimbriate bracteoles; the fruit is woody, and the numerous seeds are erect and winged. But *Eriolaena* is singular in its dense stellate pubescence, its thickened glandular petals, the absence of an androgynophore, the arrangement of the stamens in several regular ranks on the outside of the staminal tube, the numerous carpels, and the divergent stigmas.

Dumont⁽⁶⁾, investigating the anatomy of the Helictereae, found *Eriolaena* agreed with *Pterospermum* in having secretory canals ("canaux gommeux"), but these were only found in the leaves of *Eriolaena*; the bast of *Eriolaena* differed from that of *Pterospermum* in the better stratification of the fibres. Miss Chattaway⁽⁵⁾ has found "*Pterospermum*" tile cells in that genus, and "*Durio*" tile cells in two other genera of the Helictereae, *Kleinhovia* and *Reevesia*, but tile cells are lacking in the wood of *Eriolaena*. On the whole, therefore, it seems desirable to retain *Eriolaena* as a distinct tribe.

ANTHERS OF THE BOMBACACEAE

The most outstanding feature of this family is the very variable structure of the androecium, varying from almost free stamens (*Bombax insignis*) to an almost continuous tube (*Montezuma*, *Bernoullia*) with various modifications (as in *Chorisia* and *Boschia*). Bentham and Hooker⁽²⁾ expressed this heterogeneity very well in their description of the androecium, of which the following is a translation:

"Stamens numerous, hypogynous, more or less united; sometimes divided at the apex into many filaments, or into 2 to many branches each bearing one to many anthers; sometimes the short filaments being attached on the outside, or the anthers sessile; rarely the stamens almost free or subdefinite. Anthers globose, oblong, reniform, annular, anfractuous, or linear, terminal or adnate (etc.)."

This varied structure of a single feature in an otherwise homogeneous family seems deserving of special study, which will throw light upon its ecological and phylogenetic import. The best and simplest plan is to regard the anthers of the Bombacaceae as invariably unilocular; in many cases two or more unilocular anthers are borne on the same compound filament or phalanx (*Fremontia*, *Ceiba*); the filaments or phalanges are invariably united, shortly or throughout a part, or the whole of their length, to form a tube; this tube is generally adnate at the base to the base of the petals (as in *Ochroma*).

The number of phalanges varies, but they tend to group themselves in five major phalanges; this grouping may be so complete that

only five phalanges are apparent. Typically, the androecium of the tribe is *mona-penta-delphous*.

The chief evidence in support of this view is afforded by a dissection of *Bombax malabaricum* DC. Here, the staminal tube splits up into five major phalanges; each of these in turn breaks up into (a) filaments bearing unilocular anthers, (b) thicker filaments. The writer took one of these thicker filaments, and found that it bore at the summit four anther loculi; a thin translucent line ran the whole length of the filament. With a chisel-pointed needle, the anther-bearing summit was split into two, and the filament then divided easily down the translucent line, almost to the base, into two filaments each bearing two anther loculi. In turn each of these was split throughout its length into two unilocular stamens resembling those under (a) above. The thicker filaments (b) must therefore be amalgamations of the simple filaments (a). As the anthers of *B. malabaricum* measure half a centimetre, and the filaments over 5 cm., their dissection is a simple matter.

THE FREMONTIEAE

Fremontia Torrey and *Chevretonia* Humb. et Bonpl. are allied monotypic genera found in southern North America. The former is an ornamental shrub of some horticultural value, while the latter is the remarkable Mexican "Hand-flower tree," so called on account of the resemblance of the five branches of the androecium to the fingers of the hand.

Bentham and Hooker(2) placed these genera in a subtribe of their own, the Fremontieae, of the tribe Bombaceae of their Malvaceae, with the following characters: "Leaves simple, palminerved; petals absent; anthers 10, linear, adnate in pairs to the branches of the column, simulating 5 bilocular anthers." But they revised this view in the "Addenda et Corrigenda" to the same volume, and transferred the tribe Fremontieae to their Sterculiaceae, saying that the anthers were really solitary and bilocular, and not paired and unilocular. Other writers have retained them in this family, with the exception of Hutchinson(9), who refers them back to the Bombacaceae.

The proper family for the tribe clearly depends upon the structure of the androecium, and especially as to whether the anthers are unilocular or bilocular. No similar androecium is found within the Sterculiaceae. But analogous ones occur in the Bombacaceae in *Ceiba* and *Quararibea*, only here the five arms bear numerous unilocular anthers instead of only two. The androecium of *Chorisia* is indis-

tinguishable from that of *Fremontia* (at least in the species *C. speciosa* St Hil.; the material of this at Oxford and also at Kew does not agree with the drawings of this species in Engler and Prantl), except for an outer whorl of rudimentary staminodes which are only found in *Chorisia*. But Engler and Prantl(7) describe the anthers of *Fremontia* as "5 bilocular," and those of *Chorisia* as "10 unilocular"!

Chorisia has always been placed in the Bombacaceae, and its affinities with *Ceiba* and with *Bombax* itself show this to be its proper station. Therefore if the structure of the androecium forms the only ground for the exclusion of the Fremontieae, it is insufficient ground. The only other ground is the apetaly of the Fremontieae, suggestive of that of the Sterculieae, but there is no other point of resemblance between these two tribes; and apetaly occurs in the Bombacaceae in the genus *Cullenia*. The wood of *Fremontia* is described by Miss Chattaway as differing markedly in structure from that of the Sterculiaceae, and especially from that of the Sterculieae.

Therefore, the Fremontieae should be retained in the Bombacaceae, as their only affinities are with that family. Like the subtribe Durioneae of Bentham and Hooker, they have simple leaves and an epicalyx, but their palmate leaf venation and stellate (not lepidote) indumentum distinguish them from that subtribe. From all other tribes of the Bombacaceae, they differ in having apetalous flowers.

THE KYDIEAE

Kydia Roxb. The two closely allied species of this genus are forest trees confined to India. Systematically the position of the genus is obscure. Bentham and Hooker(2) placed the genus in the subtribe Abutileae of the tribe Malveae, although according to their conspectus of tribes it would be placed in their Bombaceae. They omit, entirely, to mention that the fruit is capsular, and this, together with the exceptional structure of the androecium and the bracteoles, seem sufficient to exclude *Kydia* from the Malveae. It is retained there by Engler and Prantl(7), and keyed out by them in the same section as the Australian genus *Howittia*. It resembles this in its trilocular ovary and its capsular fruit; but differs from it markedly as regards geographical range, habit, habitat, general appearance, pubescence, sex distribution, nature of epicalyx, type of petals, form of the inflorescence, structure of androecium, and type of stigma. On the balance of these features, it can hardly be a close ally of *Howittia*, which genus is a very atypical member of the Malveae.

A further point of interest is the superficial affinity of *Kydia* with two mutually similar genera of the tribe Hibisceae. These are *Dicello-*

styles, represented in Sikkim and Ceylon, and *Julostyles* with a single Singhalese species. There can be no doubt that *Kydia* belongs to the Malvaceae (in the broad sense). Therefore, since it belongs to no tribe at present constituted within that family, it must constitute a tribe in itself, which may be called the "Kydieae." It will be far better to recognise its peculiarities by thus isolating it than by assigning it to an established tribe, where its position will be an anomalous one. Owing to its capsular fruit, the Kydieae will form a part of the reconstituted family Bombacaceae.

GENERA ATYPICAL OF FAMILIES

The confusion which has arisen with regard to the taxonomy of the Malvales may be cleared up by a careful consideration of several genera which vary from the types of their respective families.

Gonystylus Teijsm. et Binn. This is a somewhat obscure genus comprising seven species of shrubs, confined to the Indo-Malayan region. In the "Nachträge" to Engler and Prantl(7), a special family, the Gonystylaceae, was created for it, and this arrangement has been retained by Hutchinson(11). It only differs from the family Scytotetalaceae in the following points: the ovules are solitary in each loculus (but two or more per loculus in the Scytotetalaceae); the fruit is several seeded and lacks endosperm. These points of difference are not very fundamental, and superficially *Gonystylus* closely resembles the genus *Scytotetalum*. Hence, it should not be set apart as a separate family, as its variations from the typical Scytotetalaceae are of hardly more than generic significance.

Prockia P. Br. ex L., four shrubs, Tropical America. *Hasseltia* H.B.K., five shrubs, Central America. The first of these two allied genera was taken by Bentham and Hooker(2) as the type of the tribe Prockieae of the Tiliaceae, but in Engler and Prantl(7), Warburg ranges them beside *Banara* and *Pinoda* in a special group "Scolopieae-Prockieae" of the Flacourtiaceae; they differ markedly from those two genera in having a valvate calyx and axile placentation. There is clearly no special advantage in Warburg's arrangement, since the group he creates is not a homogeneous one. Hutchinson retains these genera in the Tiliaceae, which is almost certainly their proper family. This applies also to *Plagiopteron* Griff., whose single species is a straggling Burmese shrub.

Christiana DC. The single species of this genus is a forest tree with an interesting distribution from Madagascar across Africa to Guiana. Bentham and Hooker, and also Engler and Prantl, place it in the tribe Brownlowieae of the Tiliaceae. Its general characters are

those of a Malvaceous plant, the numerous free stamens are indicative of Tiliaceae, and the characteristics of the calyx and the anthers leave no doubt that it is one of the Brownlowieae. In Engler and Prantl(7) it is suggested that the stamens of the male flowers are united, but in the excellent West African material examined the filaments are free, but are clustered on a conical elevation of the torus; there is no suggestion of a staminal tube; hence it is all the more likely that the genus belongs to the Tiliaceae.

The form of the fruit, however, is strongly suggestive of the tribe Sterculieae; it is of 3-5 free woody carpels, each of which contains a solitary seed; dehiscence is loculicidal, each carpel splitting into two hemispheres. The dioecious flowers also suggest the Sterculieae, but the presence of petals, which persist until the fruiting stage, definitely excludes *Christiana* from that tribe.

Grewia L. M. Burret(3) has divided this genus into three, reviving two subgeneric titles as names for his two additional genera, *Vincentia* and *Microcos*. *Vincentia* is only distinguished from *Grewia* by its "unswollen" stylar apex, and by the more numerous ovules in each loculus; these differences are so minute and difficult of determination that they can be of little practical value. But *Microcos* is readily distinguishable by its simple unlobed stigma and clobate fruit.

Whilst examining the wood structure of the Malvales, Miss Chattaway(5) found that in the genus *Grewia* (*sensu lato*), and only in that genus, were both types of tile cells to be found. The following species had the "*Pterospermum*" type of tile cell (the old name is given on the left, and that proposed by Burret on the right-hand side):

Pterospermum type

<i>Grewia multiflora</i> Juss	= <i>Grewia multiflora</i> Juss
<i>G. salvifolia</i> Roxb.	= <i>G. Rothii</i> DC.
<i>G. populifolia</i> Vahl	= <i>G. tenax</i> Aschers et Schwf.
<i>G. Rolfei</i> Merrill	= <i>G. Rolfei</i> Merrill
<i>G. tilifolia</i> Vahl	= <i>G. tilifolia</i> Vahl
<i>G. vestita</i> Wall	= <i>G. vestita</i> Wall

The following species, tabulated in the same way, were found to have the "*Durio*" type of tile cell:

Durio type

<i>Grewia paniculata</i> Roxb.	= <i>Microcos tomentosa</i> Sm.
<i>G. stylocarpa</i> Warb.	= <i>M. stylocarpa</i> Burret nov comb.
<i>G. globulifera</i> Mast.	= <i>M. globulifera</i> Burret n.c.
<i>G. latifolia</i> Mast.	= <i>M. latifolia</i> Burret n.c.
<i>G. laurifolia</i> Hook.	= <i>M. laurifolia</i> Burret n.c.
<i>G. microcos</i> Linn.	= <i>M. paniculata</i> Linn.
<i>G. miqueliana</i> Kurz	= <i>M. lanceolata</i> Burret n.c.

It will be seen that in every case the "*Pterospermum*" tile cells are found in Burret's genus *Grewia*, and the "*Durio*" tile cells in his genus *Microcos*. Only thirteen out of some 150 species of *Grewia* have been examined by Miss Chattaway so far; but these results indicate that the distinction between "*Durio*" and "*Pterospermum*" types of tile cells is of generic significance, and that Burret's genus *Microcos* is distinguished from *Grewia* by wood structure as well as by floristic structure.

Humbertiella Hochr. This new genus, which was collected by Humbert and described by Hochreutiner⁽⁸⁾ in 1926, consists of a single species of shrub, *H. quararibeoides*, found in Madagascar. Hochreutiner refers the genus to the Malvaceae, mainly on account of its membranaceous corolla, but states that it resembles one of the Dombeyaceae (Sterculiaceae).

Humbertiella agrees with the genus *Dombeya* in the following points: an epicalyx of three bracteoles; conspicuous petals; stamens united and alternating in groups with five interior staminodes; five free stigmas; and a capsular fruit. Hence it may be referred to the tribe Dombeyaceae, which tribe is predominantly Mascarene in its distribution.

Leptonychia Turcz. This genus has about eight species of small trees and shrubs in tropical Asia and Africa. Its most singular feature is the androecium; and for the sake of simplicity in describing it here, I use the term "instaminode" as denoting a staminode interior to the fertile stamens, and "exstaminode" as denoting an exterior one. Both stamens and staminodes are united at the base in a short urceolus. The five instaminodes are petaloid and alternipetalous, the ten stamens bear bilocular anthers, and are oppositipetalous, the numerous exstaminodes are filiform, and irregularly alternipetalous.

Bentham and Hooker placed the genus in the tribe Buettnerieae of their Sterculiaceae. There can be little doubt that this is its true position; the androecium is definitely that of the Buettnerieae. But apart from that, *Leptonychia* shows a striking resemblance to Burret's genus *Microcos*, which he has separated from *Grewia* in the tribe Grewiaceae of the Tiliaceae. Species of *Leptonychia* have often been referred to *Grewia*. The superficial resemblance is remarkable, and as regards its simple stigma and clobate fruit, *Microcos* comes nearer to *Leptonychia* than it does to *Grewia*. Further, *Grewia* has tile cells of the "*Pterospermum*" type in its wood, whereas both *Microcos* and *Leptonychia* have "*Durio*" tile cells. The petals of certain species

of *Grewia* (e.g. *G. mollis* Juss.) resemble those of *Leptonychia* in being thickened and glandular at the base; and suggest a transition to the highly evolved petals of *Buettneria* and *Theobroma*.

It seems probable that *Leptonychia* lies on the direct line of evolution from the *Grewieae* to the *Buettnerieae*. In some species of *Grewia*, the stamens are slightly united at the base, but are all fertile. This structure is modified in *Leptonychia* by the greater connation of the filaments, and the sterilisation of the redundant exterior anthers.

Howittia F. v. M. This genus has a single species, *H. trilocularis*, an Australian shrub. Bentham and Hooker(2) placed it in the subtribe *Abutileae*, of the tribe *Malveae*, of the *Malvaceae*; but it appears to be out of place there. Firstly, its habit is not herbaceous, but shrubby. Then, the definition of the tribe *Malveae* involves the following points: staminal column antheriferous at the apex; stylar branches as many as carpels; ripe carpels seceding from the axis. But in the specimens of *Howittia* at Kew, the staminal column is antheriferous almost to the base, there are no stylar branches, and the carpels of the capsular fruit remain attached at the base, to the axis. *Howittia* must therefore belong to some other tribe of the *Malvaceae*, and may best be assigned to the *Hibisceae*, in which the staminal column is antheriferous throughout its length, and the loculicidally dehiscent carpels do not secede.

Hampca Schlecht. This genus comprises three species of Central American trees. Bentham and Hooker(2) refer it to their *Bombaceae*, but remark that this is only on account of the staminal column, as the pollen is that of the *Hibisceae*, and suggest affinities with *Thespesia* (*Hibisceae*). They describe the staminal column as short, dividing into long filaments. In the specimen dissected, the staminal column was long, bearing *throughout its length* numerous short filaments. This was *H. trilobata* Standley, which is a recently discovered species. But the presence of three small bracteoles in all the species makes the genus atypical within the subtribe *Matisieae* of the *Bombaceae*, to which Bentham and Hooker assigned it.

If it be transferred to the *Hibisceae*, *Hampca* will come nearer to its true systematic position; and this instance suggests that the tribes *Bombaceae* and *Hibisceae* (of Bentham and Hooker) are more closely allied than is generally supposed.

Bernoulia Oliv. This arborescent, monotypic Central American genus appears to have been referred by Oliver to the *Sterculiaceae*. In Engler and Prantl(7), it is placed in the tribe *Matisieae* of the

Bombacaceae; but this tribe is distinguished by its simple leaves, whereas those of *Bernoullia* are five-digitate.

The proper family for this genus depends on the interpretation put upon the structure of the androecium. The anthers are so clustered and contorted together upon the apex of the column, that it is virtually impossible to say whether they are unilocular or obscurely bilocular. But they show a tendency to group themselves in five bundles suggestive of the Bombacaceae, and especially of *Ceiba*, which also has digitate leaves. The proper tribe for the genus, then, is the Adansonieae of the Bombacaceae; this tribe is well represented in Central America.

EXCLUDED GENERA

The following genera have been excluded entirely from the present consideration of the Malvales; because, for the reasons given in each particular case, their assignment to this order is based upon a conception of it too wide to be practicable, or is only tentative. The family Chlaenaceae has been excluded because its imbricate calyx suggests stronger affinities with the allied order Guttiferales.

Hua Pierre ex De Wild. The first description of this small genus from the Congo assigned it to the Sterculiaceae with no affinities whatsoever. In the fourth "Nachtrage" to Engler and Prantl(7), a special tribe, the Hueae, is created for it, and although the remark is made that the genus is completely isolated within the family, this tribe is inserted between the two tribes Hermannieae and Buettnerieae, both of which are typical!

The hairs of *Hua* are simple, not stellate, and after examining a specimen, and also Pierre's drawings, I fail to see how it can come within the Malvales. Superficially, the plant resembles *Gymnosporia* (Celastraceae).

Nettoa Baill. In Baillon's(1) paper on "The genus *Nettoa*, and the characters which separate the Tiliaceae and the Bixaceae," he reaches the conclusion that to exclude *Nettoa* from the Tiliaceae on account of its parietal placentation (the only exceptional feature) is to draw too artificial a distinction. Bentham and Hooker(2), and Engler and Prantl(7), include *Nettoa* in the Tiliaceae (tribe Tilheae); but Burret(3), who treats only of that family, considers it a "genus dubium." The parietal placentation should exclude it from the Malvales, and suggests that it may prove to be one of the Flacourtiaceae (cf. *Scolopia*).

Ropalocarpus Boj. must be excluded from the Malvales, owing to its strongly imbricate calyx; it may, perhaps, belong to the

Flacourtiaceae, to which family it is tentatively referred in *Die Pflanzenfamilien* (7).

Dialycarpa Mast. According to the first "Nachträge" to Engler and Prantl, the single species of this genus is really a *Brownlowia*, *B. beccarii* (Mast.) Pierre.

Neotlessmannia Burret (3). Owing to its inferior ovary, this genus is utterly atypical, and must be excluded from the Malvales; it is probably allied to *Banara* (Samydaceae).

Thurberia A. Gray. This genus has been sunk in *Gossypium*, of which it may be a synonym. The name has been adopted by Bentham for a genus of the Gramineae; and several changes in nomenclature would have to be made if the Malvaceous genus were to be restored.

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(To be concluded)

DIFFERENTIATION OF PROTOPHLOEM IN THE ANGIOSPERM SHOOT APEX

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(With 5 figures in the text)

INTRODUCTION

THE experiments of Curtis⁽³⁾ lend strong support to the view that the upward translocation of foods, like the downward, is also through phloem. When a shoot is ringed and the part above the ring defoliated, that part makes practically no further growth. Curtis' researches lead one to ask just how far up the shoot the phloem functions. To answer this question one has to ascertain first the condition of this tissue at the developing shoot apex. The vigorous growth of the superficial meristem at the non-green shoot apex giving rise to new leaves and internodes means that the necessary supplies are reaching it from below and at a rate that can maintain such growth. It is true that the superficial meristem cannot receive its supplies *immediately* from the vascular elements, for between the canopy of embryonic cells and the ends of the vascular tract there exists a gap. This gap is bridged by the forerunner of the vascular tract, the procambial strand. The question whether the elongated cells of the procambium function as a continuation of the vascular elements or not is beyond the scope of the present paper. We are going to present below some observations on the differentiation from the procambium of the earliest tissue to which the function of food conduction is ascribed, i.e. the protophloem.

LITERATURE

Our knowledge of the protophloem is still vague. In fact we could not find any important report on it since the researches of Léger⁽⁶⁾ and Chauveaud⁽²⁾. Though in their publications these authors do not employ the term protophloem, it is evident from their descriptions and figures that they deal mainly with this tissue. Their observations, however, were obviously based on material sectioned by hand, a method which leaves much to be desired in detailed developmental

studies. In their book, Eames and MacDaniels state that "in many cases—and perhaps always—it [protophloem] consists of phloem parenchyma only, sieve tubes being absent" (11, p. 90). It is difficult to see how a tissue can be designated as phloem if the characteristic sieve tube is wanting. Their statement shows clearly how slight is our knowledge about this tissue.

MATERIAL AND METHODS

The following account is based on *Tropaeolum majus* L., but stem tips of sunflower, white mustard and garden pea were also examined. Serial paraffin sections, both longitudinal and transverse, were prepared and stained either in safranin and light green or methylene blue. Freehand sections of fresh material were also examined.

THE DEVELOPMENT OF THE SIEVE TUBE

It may be pointed out at the start that all species examined show sieve tubes in the protophloem. It is also the only type of element studied in *Tropaeolum majus* L., for we did not find any companion cells. The cell that differentiates from among the outer cells of the procambial strand is directly transformed into a segment of the sieve tube without further longitudinal division. As to the parenchyma cells of the protophloem, they have no distinguishing features; they are so designated solely from their proximity to the sieve tubes.

The wall. The earliest visible differentiation of the sieve tube is the change in the appearance of the cell wall. An outer cell in the procambial strand can be picked out from among its neighbours by its much thicker and glistening wall. This stage of development is the "differentiation nacrée" of Léger, corresponding approximately to the maximum differentiation of Chauveaud. These two writers have made extensive observations on this stage (2-6). The thickened wall stains more deeply in light green and, according to Léger, has a general affinity for acid dyes. Though the author in question states that it is indifferent to basic dyes, we find that methylene blue is a good stain for it, in that the "nacré" walls stain violet, whilst the walls of the neighbouring cells stain purplish blue (walls of xylem elements stain an azure blue). This is undoubtedly due to the polychrome property of the dye.

In the fixed and dehydrated material, the "nacré" wall has lost much of its thickness, but retains its staining qualities in light green and methylene blue, thus enabling it to be traced with ease in paraffin sections. Later as the sieve tube matures, wall thickness and the

glistening appearance diminish, but the wall still stains deeper than those of the surrounding cells.

As a rule, a cell with "nacr " walls either possesses already a sieve plate or is in direct continuity below with cells possessing sieve plates. We can regard the "nacr " differentiation, therefore, as a developmental phase of the sieve tube. Only in rare cases do we find a parenchyma cell or a linear row of a few such cells possessing "nacr " walls and not leading to a sieve tube. In those cases such cells are always found next to a sieve tube.

The cell contents. When the "nacr " differentiation takes place the contents of the cell show no change. In outline the cell remains angular. Occasionally the outline is somewhat rounded or the protoplast in the fixed material shows considerable shrinkage, indicating vacuolation of the protoplast. The nucleus appears intact at this stage (Fig. 1).

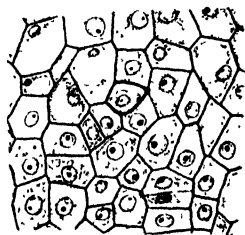


Fig. 1. Transverse view of a portion of a leaf primordium showing a cell of the protophloem in "nacr " differentiation. $\times 750$.

The sieve plate. Chauveaud describes and figures a sieve plate in his work on Angiosperm roots(2). The presence of sieve plates in the protophloem of the Angiosperm shoot is less well established. L ger in his monograph(6) merely mentions this structure and does not give detailed descriptions or figures. In all species we have examined (*Tropaeolum majus*, *Helianthus annuus*, *Pisum sativum*, *Brassica*, *Sinapis alba*), we found, as pointed out above, sieve plates in the earliest phloem elements formed from the procambium, though they can be clearly made out only under a 2 mm. immersion objective.

In *Tropaeolum* the sieve plate in the protophloem is transverse or somewhat oblique. The first sieve plate can be seen three or four cells after the beginning of "nacr " differentiation. L ger says that "nacr " differentiation and sieve plate formation synchronise (6, p. 57). In *Tropaeolum* we find that the latter lags a little behind. Though allowance must be made for developmental differences in

different species, it is probable that the little lag in sieve-plate formation has escaped Léger's notice. This is very natural, for one cannot expect that in freehand sections a distance of three or four cells would be noticed.

Sieve plates stained particularly well in methylene blue. In surface view, the sieve pores appear as almost colourless spots on a purplish blue ground (Figs. 2*a* and 3*a*). They are very small. In a sieve plate 5μ in diameter, there is a row of ten sieve pores. It is only $\frac{1}{2}\mu$ from the centre of one pore to that of the next. They are, therefore, just above the limit of the microscopic resolving power. In fact, it cannot be ascertained from the surface view whether these spots are perforated or not. In the sectional view of the sieve plate, however, one sees the plasma strands connecting the adjacent proto-plasts through the sieve plate.

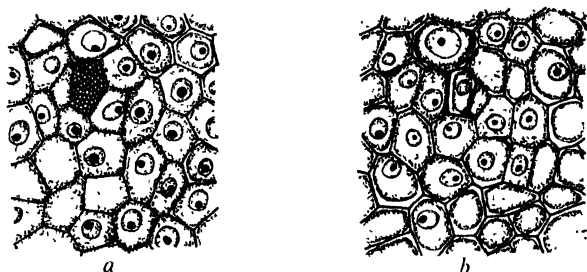


Fig. 2 *a*, a portion of a young petiole showing a sieve plate. $\times 750$ *b*, the same as seen in the next section below *a*, showing the presence of a nucleus in a sieve cell below the sieve plate shown in *a*. $\times 750$.

When sieve plates are differentiated, the cells are still very short. For example, in one case the first six plates of one tube occur in eleven transverse sections of 10μ in thickness, averaging therefore about 20μ apart. The segments in maturer parts are much longer. In a tube in a young petiole, the sieve plates are about the following distances apart from each other: 100, 80, 60, 80 and 40 micra. It is characteristic of the proto-elements that they elongate considerably after differentiation.

The degeneration of the nucleus. The prevailing opinion based on studies on secondary phloem, principally by Wilhelm(10) and Strasburger(9), is that the disappearance of the nucleus in any sieve-tube segment synchronises with its sieve-plate formation. Zacharias(11) and Schmidt(7) report, however, that the nucleus persists after the sieve plate is well differentiated. In *Tropaeolum*, owing to the narrow

lumen (seldom over $5\ \mu$ wide) of the protophloem sieve tube and the slender nucleus (about $2\ \mu$ in cross section), it is difficult to ascertain the condition of the nucleus. In one case, however, the nucleus had definitely disappeared above the first sieve plate, whilst in another case we could clearly see the nucleus after each of the first three sieve plates. The nuclei appear unaltered except that they stain more faintly (Fig. 2*b*). To answer the question as to what is the usual time of nuclear degeneration—whether concomitant with the appearance of sieve plates or after it—further observations are needed.

Collapse of the sieve tube. Léger⁽⁶⁾ and Chauveaud⁽²⁾ have observed a number of cases of the disappearance of the protophloem sieve tube either by crushing or by atrophy. As the cells get progressively older in the downward course of a tube (cf. p. 26), an attempt was made to trace single sieve tubes down their course, with the hope of determining just at what node or internode a given sieve tube begins to disintegrate. But the task proved more difficult than had been expected. It is easy to follow the sieve tube down the young petiole, but at the node its course takes a sharp bend, so that one is presented with a very oblique view of the tube, which makes it difficult to ascertain whether any change has taken place in it. Furthermore, the nine strands in the petiole merge to form three leaf-trace bundles, thus making it difficult to identify the sieve tubes in them. Nor is this difficulty obviated in the very young leaf primordium with as yet a single sieve tube, for as its procambial strand enters the stem it combines with another strand from an adjacent leaf, and this process of coalescence of procambial strands is repeated at each node as fresh leaf traces enter. As a result, sieve tubes which occur in separate leaves converge in the stem and finally lie side by side. This situation, as pointed out above, makes the identification of individual tubes difficult and uncertain.

The problem was attacked from another angle with better success. We examined the successive leaves in order to see in which one we could detect the beginning of sieve-tube degeneration. To such studies the long petiole of *Tropaeolum* lends itself. In the petiole of the seventh youngest leaf, a second sieve tube, inside and next to the first one, has developed in the most proximal procambium strand (the order of development of protophloem elements will be described in a later paragraph). In the petiole of the eighth leaf we begin to meet the sieve tubes in a semi-collapsed condition. Here the three distal procambium strands (cf. p. 27 and Fig. 5) possess two sieve tubes each and the outer one of each of them is more or less degenerated.

The sieve plate, resists crushing the longest, and in the early stage one sees no change in it. Away from the sieve plate the lumen gets smaller and smaller, reaching the smallest diameter at about the middle of the cell. In one carefully studied case, the two sieve plates of a cell are 240μ apart. At 140μ from the upper plate the lumen is entirely obliterated; the cross-section of the cell is here represented by a dot only (Figs. 3 *a*, *b*, *c*). The tube is not flattened but the lumen simply gets gradually smaller until it vanishes.

As the sieve tube first appears in the third or fourth leaf primordium and begins to degenerate in the eighth, the life of the tube—at least the ones first formed in the petiole—is about four or five times as long as the time required for the formation of a new leaf primordium from the growing point, or, to use the succinct term, its life lasts 4–5 plastochrones (cf. Askenasy(1), Schüepp(8)).

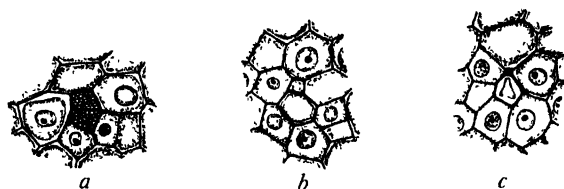


Fig. 3 *a*, *b* and *c*. Transverse views of portions of a young petiole at three different levels showing an outer sieve tube partially degenerated and an inner intact one. $\times 750$.

THE PROTOPHLOEM STRAND

The outermost cell or the next inner one of the procambial strand is differentiated into a segment of the earliest sieve tube. The order of development is acropetal. As a consequence of this mode of development, each segment of the sieve tube is younger than the one below it, and the differentiation reaches a leaf primordium at a higher level later in time than one farther away from the apex. Griffiths and Malins(5) find that, in the decussate plants they examined, the youngest protophloem element occurs in the third or fourth pair of leaves (but in *Pisum* they find protophloem already in the youngest leaf primordium). In *Tropaeolum* protophloem is differentiated as far up as the third or fourth leaf primordium. The protophloem strand which, judged by its position, is to supply the next leaf above, has reached at this time only a lower level and would probably advance to a corresponding point in its leaf in the interval of another plastochrone (Figs. 4 *a*, *b*).

The course of the protophloem strand in the petiole and down the stem is touched upon in an earlier paragraph. Here we shall describe the strands in the leaf. Each leaf primordium is at first supplied with a single sieve tube. It occupies a median position in the lamina and an abaxial one in the petiole. Lateral strands de-

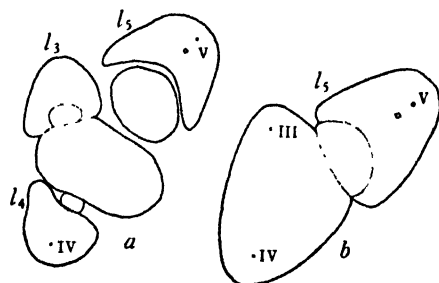


Fig. 4. *a*, transverse view of a shoot apex at the level of insertion of the third youngest leaf. *b*, the same, 0.12 mm lower, at the level of the upper end of sieve tube III, which, judged by its position, would later differentiate into the third leaf. The dots represent sieve tubes; the squares, vessels.

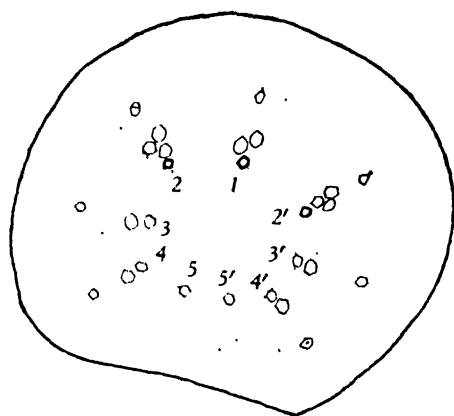


Fig. 5. A diagrammatic transverse view of a young petiole illustrating the order of development of xylem and phloem in each procambium strand.

velop first in the bundle on the abaxial side and then in succession in the bundles nearer the axis. This mode of development is illustrated somewhat diagrammatically in Fig. 5. It represents a transverse section of the petiole of the eighth youngest leaf. In each of the three distal procambial strands (1, 2 and 2') two sieve tubes have been differentiated, and the outer tube in each is in a state of

degeneration. Strands 3 and 3' possess as yet a single tube each. In strands 4 and 4' the sieve tubes are distinguished by the "nacr " walls of the cells, which, however, still retain their nuclei. Sieve plates in them are found only in the lower part of the petiole. In strands 5 and 5', there is as yet no sign of phloem differentiation.

Mention may be made of the development of the protophloem with respect to that of protoxylem. The earlier sieve tubes of a young leaf precede the tracheal elements of the same procambial strand; the first sign of vascular differentiation in the leaf primordium is the thickening of the walls of the embryonic sieve tube. The later formed strands of the protophloem of a leaf, however, lag behind their accompanying protoxylem strands in differentiation. In Fig. 5, for instance, in the two adaxial procambial strands (5 and 5'), there is as yet, as pointed out above, no suggestion of protophloem differentiation, but the xylem cells can be picked out by their big lumen and highly vacuolated contents, though they have not lost their protoplasts nor have they acquired thick walls. In other words, the development of the protoxylem in different strands of a leaf follows in closer sequence in time than is the case with the protophloem.

SUMMARY

Protophloem of *Tropaeolum majus* consists of sieve tubes, the plates of which possess extremely fine sieve pores.

"Nacr " differentiation precedes sieve-plate formation.

The nucleus of the sieve-tube cell segment degenerates either simultaneously with the sieve-plate formation or after it.

As is characteristic of protoxylem and protophloem elements, the elements of a sieve tube in the protophloem lengthen greatly during their differentiation.

The sieve tube begins to collapse after an interval which equals, when measured in terms of leaf formation, 4-5 plastochrones. After the second tube is fully differentiated, the first is found in a state of degeneration.

I am deeply indebted to Prof. Priestley for his suggestions and advice and for the use of his laboratory facilities. Thanks are also due to Miss Lorna Scott who kindly turned over to me some material which she had collected and fixed.

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INVESTIGATIONS ON THE DEVELOPMENT OF ROOT HAIRS

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(With Plate I and 19 figures in the text)

INTRODUCTION

SINCE the discovery of root hairs by the microscopists of the seventeenth century their interest to botanists has been evidenced by a long series of publications. Indeed in the higher plants one could hardly find another cell that lends itself so well to investigation. Root hairs can conveniently be made to grow in a variety of media, and their superficial position on the smallest roots is most convenient when microscopic observation of living, developing cells is desired. An additional interest is provided by their intimate connection with the phenomena of absorption.

Miss Snow's paper⁽²⁹⁾ includes a satisfactory review of the work done on root hairs prior to 1905, the date of its publication, and her bibliography contains eighty-nine titles. The opinions of earlier investigators may be summarised as follows: There was conflicting evidence as to the effects of light and darkness. The importance of temperature had been noted, but without any definite statement as to its action. Conflicting reports as to the effects of contact with solids had been brought forward, but it was known that in certain water plants hairs were produced only on roots penetrating the mud. There was a rather general but not unanimous agreement that retardation of growth of the root led to the production of more hairs, and immersion in water had been found in some plants to inhibit their production.

During the present century the work has continued, with the result that a mass of data has accumulated, often giving rise, as in the earlier period, to conflicting conclusions.

A number of workers have turned their attention to abnormalities of root-hair growth under various more or less abnormal conditions. Stiehr⁽³¹⁾ obtained various types of abnormal growth by such treatment, and Hill⁽¹⁹⁾ found branched and swollen hairs to be normal in certain marsh plants. Farr^(10,12) found that in *Georgia* collards such

abnormal hairs were produced in weak solutions, in solutions near the acid and alkaline limit for the growth of hairs, and when roots had been placed in solutions after growth in air had begun.

With respect to the conditions favourable for root-hair production many observations and opinions have been reported. Miss Snow found high temperatures unfavourable, and lack of oxygen even more so. She noted also the normal absence of hairs on roots of *Zea mays* grown in water. In her opinion retardation of vertical root growth, by mechanical or other means, was an important factor in root-hair production. Miss MaGowan(25), Hansteen Cranner(17, 18), Kisser(23), Farr(6, 7, 8, 11, 13), Mrs Farr(14, 15, 16) and Sorokin and Sommer(30) presented evidence that the presence of calcium is an important condition for root-hair development. Mevius(26), on the contrary, decided that in Indian corn the presence or absence of root hairs is not related to the presence or absence of calcium in the solution, and Pearsall and Wray(27) found that root hairs of *Eriophorum angustifolium* tend to be longer and more abundant in solutions of low calcium content and high basic ratio. The importance of hydrogen-ion concentration was emphasised by Farr. Acid solutions, he found, had an inhibiting effect, which could, however, be overcome within limits by increasing the concentration of calcium(8, 9, 12). In strongly alkaline solutions root hairs were poorly developed also, and the cortex was often ruptured.

The composition of the cell wall has also received its share of attention. Stiehr(31) inferred from his experiments that the end wall of the hair was composed of softer materials. Miss Roberts(28) was in agreement with this, and distinguished in the wall of the hair an outer layer of calcium pectate and an inner one of cellulose. Miss Howe(21) stated that the outer layer was of pectic material, but the inner one of callose. Ziegenspek(34), on the other hand, found an inner layer of cellulose, replaced by amyloid at the tip, and believed that the presence of the plastic amyloid made possible the growth of the hair. Hopmann(29) cast doubt on this hypothesis, reporting, in 1931, the absence of amyloid from root hairs developed in water, and the presence of cellulose over the whole inner surface under all conditions. In the meantime Miss Addoms(1) had reported her inability to demonstrate satisfactorily the presence of either cellulose or pectic material.

A number of earlier writers had found in certain plants special short cells interspersed among the other cells of the epidermis and devoted to root-hair production. Leavitt(24) in 1904 studied this

aspect of the subject in some detail, finding such cells only among the higher cryptogams, certain monocotyledons, and a few water plants. Among the later investigators Bartoo(2) noted this peculiarity in his study of *Schizaea rupestris*.

Two general theories as to the mechanism of root-hair production have been brought forward. One, suggested by Miss Snow, and concurred in by Jeffs(22), postulates that when the vertical elongation of the epidermal cell is checked either by cessation of growth on the part of the inner cells, or by external mechanical means, the cell extends horizontally to form a hair. The second, advocated by Miss Roberts, and with many adherents among later authors, suggests that pressure from within the cell, acting on a softer spot in the cell wall, is responsible. One weakness of the former hypothesis lies in its failure to account for the fact that, while some epidermal cells produce hairs, others in the same region of the root remain hairless. The adherents of the latter theory are still in disagreement as to the exact difference between the hypothetical soft area and the rest of the cell wall, and no one has suggested the proximate cause of this difference.

The present investigation is an attempt to add to our knowledge of root hairs by further cultural and microchemical studies. Various varieties of *Brassica oleracea* L. were at first used as the experimental material, all giving consistently similar results, but finally seedlings of Chinese cabbage (*Brassica napus* L. var. *chinensis* (L.) Schulz) were selected owing to their vigour of growth and the small diameter of their roots. Later, other plants were used in checking up the hypotheses that grew out of the experiments.

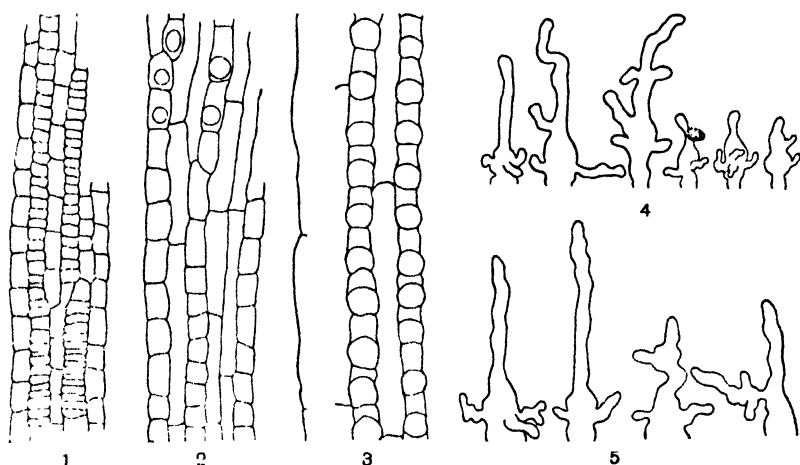
NORMAL ROOT-HAIR DEVELOPMENT

Normal root hairs were found to develop at room temperatures in moist air and in tap water. In the latter case development was more vigorous if small amounts of calcium salts were added.

The epidermis of roots of *Brassica* species was found to exhibit peculiar morphological features, which so far as the writer is aware have hitherto not been recorded. It is made up of two kinds of cells, long and short, easily distinguishable throughout the length of the root. The short cells are less specialised in appearance than those discovered in other forms by earlier writers and are arranged in distinct rows.

Text-figs. 1, 2 and 3 (p. 33) represent strips of epidermis separated from the roots after warming with dilute ammonia to dissolve the pectic acid cement between epidermis and cortex. Text-fig. 1 is

from the meristematic region just above the root cap. Even here the two types of cells are easily distinguishable. The short ones, owing to their rapid division, are wider than long. At this stage they are still full of protoplasm, while the long cells have already begun to vacuolate. Text-fig. 2, further from the tip, illustrates the upper part of the region of elongation. The short cells, unlike the long ones in adjacent rows, have continued to divide, and although they have elongated somewhat, the disparity in length between them



Text-figs. 1-3. White mustard root; surface view of successive regions of the epidermis near the root tip, showing rows of "long" and "short" cells. $\times 110$

- 1 Meristematic region, divisions in long cells have ceased
2. Region of elongation and region of first formed papillae
- 3 Haired region, the circles represent bases of hairs

Text-fig. 4. Chinese cabbage root, a few abnormal hairs developed in standing tap water. $\times 90$.

Text-fig. 5. Chinese cabbage root, a few abnormal hairs developed in flowing ammonium oxalate solutions. $\times 90$.

and the long cells is even greater than in Text-fig. 1. At the top of Text-fig. 2, five of the short cells have begun to produce root hairs and the bases of the papillae appear as circles on their surface. Text-fig. 3 is from the haired portion of the root. Every short cell has produced a hair. The position of the hair is invariably near the distal end of the cell, and when the cells are particularly short, as in Text-fig. 3, the hairs are at the extreme end, often projecting over the cell below. The rows of long cells are hairless.

Plate I, fig. 1, is from a transverse section of a cabbage root through the meristematic region. The small, transparent, external cells belong to the root cap. In the epidermis the short cells are clearly distinguishable. They are full of protoplasm and the nucleus is prominent. Their position, each in contact with two cortical cells, also serves to differentiate them. The long cells are narrower and have begun to vacuolate. The cortical cells appear larger in transverse section and have already begun to round up, leaving air spaces, while the cells of the stele are narrow and closely packed. Above the plane of this section, a region of elongation of 3 mm. or less in length precedes the piliferous region.

Usually the stockier roots are more densely haired. The roots taper gradually to the tip, but frequently distinct swollen regions are observable. Normally root hairs are abundantly developed but they are not always uniformly spaced. Often there is a zonation in the piliferous region. A zone of normal, hair-producing short cells is followed by one where all the cells are more elongated, though hairs are just as numerous, and then by a region of still longer cells, and sparse hairs. This region may again be succeeded by a second short-celled, densely haired zone. The zonation is variable from root to root and is often absent. Some measurements from a root growing in flowing tap water will illustrate it. In the densely haired region at the top, the long cells measured 330-440 μ , the short cells 90-135 μ . Further down, the long cells measured 585-900 μ , and the short cells, all still forming hairs, 190-276 μ . Below this, the short cells measured up to 300 μ , and the majority were hairless.

The microchemistry of the cell walls in the developing region provided facts of considerable interest. In the zone of division the cell walls are very thin, but even here give a clear colour test for cellulose, with iodine and 65 per cent. sulphuric acid. A preliminary warming in 1 per cent. potassium hydroxide or 2 per cent. ammonia is desirable to remove protein and fat from the walls. This treatment is unnecessary except in the case of the meristematic cells. Unless the iodine solution penetrates thoroughly the test is unreliable. To ensure such penetration, the material, in small Syracuse watch glasses, was soaked in iodine potassium iodide solution for 15-30 min. The roots, stained brown by the iodine, but with as yet no trace of blue colour, were then placed on a glass slide, and the excess iodine having been removed, were covered with a drop of 65 per cent. sulphuric acid. A clear blue colour in the walls always resulted, leaving no doubt as to the presence of cellulose.

Hot water produced no maceration, but after heating for 30–60 min. in 2 per cent. ammonia the tissue separated almost to the region of root-hair formation, first into long rows and then into individual cells. After this treatment the remainder of the cell walls proved to be cellulose, dissolving completely in copper oxide ammonia. The cementing substance was clearly pectic acid, as denoted by its solubility in 2 per cent. ammonia. If it had been pectin the cells would have separated in hot water. If calcium pectate had been present, it would not have dissolved without previous treatment with acid. Corroboration of the pectic nature of the primary wall was shown by the strong cherry-red staining with ruthenium red. The above results are in exact agreement with the statements of Tupper-Carey and Priestley(33) concerning the nature of the walls of meristematic cells.

In the region of the first formed papillae, differences between the primary walls of long and short cells were demonstrated. The roots were placed in 2 per cent. ammonia in large test-tubes and heated for about 30 min. in a water bath set at 80° C. This temperature was sufficient to remove any pectin and pectic acid, without causing tangling and collapse of the root hairs. The middle lamella of the cortical cells having thus been partially dissolved, thin strips of epidermis could be removed by means of a sharp needle. Such strips were stained in ruthenium red or methylene blue and mounted in dilute ammonia with the haired surface uppermost. A few drops of concentrated ammonia were run under the cover-glass and this was followed in turn by a gradually increasing concentration of active copper oxide ammonia. By careful manipulation, the solution of the cellulose in the walls was brought about without mechanical damage to the frail wall structure remaining. The walls of the long cells up to the region of well-formed, actively growing hairs dissolved completely, the solution and debris flowing in channels between the rows of short cells which, including the first papillae and two or three cells directly below them, remained intact in chains. After this treatment the outer and end walls of the short cells appeared more distinct and thicker than the inner walls, which usually became very thin, and often dissolved, as did also the walls of all cortical cells in the same region. Obviously a change in the pectic layer of the short cell walls had taken place. This change was one from pectic acid to calcium pectate, for when the treatment was preceded by a half-hour of heating in 2 per cent. hydrochloric acid at 80° C. all the cell walls in this region, including those of the young hairs, dissolved.

The results of microchemical tests on the walls of the young growing hairs themselves merit special consideration. The blue colour test for cellulose with iodine and 65 per cent. sulphuric acid in the young growing hairs indicated a thin layer of this substance continuous with the wall of the epidermal cell itself, extending along the sides of the hair and over the tip. The colour was comparatively faint and very often disappeared rather rapidly as hydrolysis proceeded. It was always obtainable, however, while the iodine test for amyloid was always negative. After dissolving any pectic acid in 2 per cent. ammonia, and mounting in copper oxide ammonia to dissolve the cellulose, some of the tips of the very young hairs and of the papillae dissolved completely. There is no doubt as to the correctness of this observation. In some cases the actual process of their solution and disappearance was observed. With just as much certainty it can be stated that others did not dissolve, though the remaining wall was very thin and frail, as evidenced by the fact that a disturbance which had no effect on the side walls would distort the wall of the tip. Further proof of the more yielding nature of the tips was apparent when roots that had been growing in a concentrated calcium salt solution were transferred to distilled water on a slide. Under these conditions the hairs burst, and the bursting, as in the hairs with which Stiehr worked, took place always at the tips. All evidence pointed to the conclusion that this increased plasticity was due to a difference in the pectic layer of the cell wall. No evidence was obtainable of any difference between the cellulose layer at the tip and that along the side walls.

In the older, full-grown hairs the iodine and sulphuric acid test revealed a thicker layer of cellulose along the sides and at the tips. Moreover, the blue colour was retained after several hours in the 65 per cent sulphuric acid, indicating that complete hydrolysis had not taken place. That the calcium pectate layer was also more firmly fixed was shown by the fact that it was necessary to heat the roots for a much longer period in 2 per cent. hydrochloric acid before this wall would dissolve in 2 per cent. ammonia. Often long pieces of mature hairs were seen to remain after all other cell walls outside the endodermis had dissolved in copper oxide ammonia following this treatment. In such cases it was the pectic part of the wall that was left undissolved, for when the time of preliminary heating in 2 per cent. hydrochloric acid was increased complete solution followed. The difficulty of dissolving these older hairs may be explained by the presence of a more strongly calcified pectate, or of a pectocellulose

complex such as the "protopectin" of Sucharipa (32). If "protopectin" is present it may at least be said that the whole wall has not been transformed into this substance. Thin transverse sections of root hairs cut in paraffin were treated with iodine and 65 per cent. sulphuric acid. When viewed with an oil-immersion lens of sufficient resolving power, they showed clearly an inner blue layer of cellulose and an outer yellowish or almost colourless layer of calcium pectate.

As a preliminary to the consideration of the next section, it will be convenient to recapitulate the points already established. The root-hair wall is composed of an inner layer of cellulose with an outer one of pectic substance and possibly an intermediate region of "protopectin" in the older hairs. The cellulose of a growing hair differs from that of a mature one, being soft, and more easily hydrolysed by acid. No difference is evident between the cellulose at the tip of the hair and that lining the sides. The pectic wall of the sides of the hair and of the epidermal cell on which a hair is growing is composed of calcium pectate. At the tip of the growing hair, it is sometimes composed of pectic acid and sometimes partly at least of calcium pectate. In the latter case, however, the wall covering the tip is soft and more delicate than that of the sides. This, and the fact that the growing hair is always turgid, is in accordance with the theory of root-hair growth suggested by Miss Roberts. The long cells that produce no hairs have walls similar to those of the hair-producing cells, except that in the region where hairs are initiated they have no calcium pectate, but only cellulose and pectic acid.

EFFECTS OF CALCIUM DEFICIENCY

Experiments were carried out to study the origin and subsequent development of root hairs in solutions deficient in calcium.

Seedlings, with roots 3-5 mm. long, were placed on thin sheets of cork, coated with paraffin and measuring 6.5×3.5 cm., the roots hanging vertically downwards through holes in the cork into a solution on which the cork floated. Generally about fifteen seedlings were placed on each piece of cork.

As already stated, the seedlings grew well in tap water, and produced apparently normal root hairs. After some time in the water, however, a change was noticeable. The later formed epidermal cells obtained a greater length than normal, and the hairs produced were shorter. After 4-8 days, if the water was left unchanged, only stunted abnormal hairs developed. All such hairs were inflated, bottle-shaped, bulbous at the base, or variously branched. Camera lucida drawings

of such hairs are shown in Text-fig. 4 (p. 33). The abnormalities strongly suggest the action of internal pressure on plastic areas in the cell wall. Microchemical tests showed the walls of these hairs to be similar to those of normal hairs in that they consisted of an inner layer of cellulose with an outer layer of calcium pectate.

Notwithstanding the microchemical evidence, it was thought advisable to investigate the possibility that lack of calcium was the cause of these abnormalities. That the solution of the problem lay in this direction seemed much more probable as a result of some experiments carried out *in vitro* with pure pectic acid, and its reaction with calcium salts. The pectic acid was obtained from commercial, powdered, citrus pectin, and purified by dissolving in potassium hydroxide solution, reprecipitation by concentrated hydrochloric acid, and careful washing. Such pectic acid, dissolved in dilute ammonia, united with the calcium of a solution of calcium sulphate to form a pectate insoluble in 2 per cent. ammonia, but the product was softer and more plastic when the amount of calcium added was insufficient to neutralise all the acid radicals of the pectic acid molecule. This softer substance would seem to be calcium pectate with each molecule of pectic acid only partially neutralised, rather than a mixture of calcium pectate and pectic acid, since an excess of ammonium hydroxide solution, in which pectic acid is easily soluble, was always present. Since a molecule of pectic acid has four acid radicals, four successive stages would seem to be possible in neutralising it to form calcium pectate, and these might be passed over quickly or allowed to succeed each other slowly, depending on the conditions of the reaction.

To test whether the abnormal root hairs above mentioned might be the result of the using up of available calcium, seedlings were allowed to grow in tap water to which small amounts of ammonium oxalate had been added to precipitate part of the calcium. To dishes holding 60 c.c. of tap water were added 5, 10, 15, 20 and 25 drops respectively of a 1 per cent. solution of ammonium oxalate, and seedlings were floated on corks in the water so treated. In all cases normal hairs were produced at first, but branched hairs succeeded normal ones in the solutions more rapidly than in the case of controls in tap water. Individuality was exhibited by the roots, some individuals forming more branched hairs than others, but the amount of abnormality increased consistently with increasing amounts of ammonium oxalate solution, until at the end of the series both root and hair development were retarded. While this result is favourable

to the hypothesis that an insufficiency of calcium causes the production of abnormal hairs, it also indicates that internal conditions have a bearing on their production, for in even the 25-drop solutions some of the roots formed normal hairs.

The view that abnormalities are caused by a deficiency of calcium ions is further strengthened on examination of seedlings grown in Knop's solution without calcium. In comparison with roots grown in the complete nutrient solution they were unhealthy and shorter. The hairs were often densely developed at the top but succeeding hairs were found to be swollen and variously branched. Moreover, occasionally near the tip of the root, many swollen epidermal cells were formed among the short hairs. In brief the abnormalities produced in Knop's solution without calcium were similar to but more pronounced than those in the tap water to which the larger amounts of ammonium oxalate had been added. The individuality mentioned above was also in evidence here, some roots forming normal hairs while the neighbouring ones were pronouncedly abnormal.

Two possible explanations for such individuality present themselves. Some seeds may contain more calcium than others, or the calcium they contain may be more available for the formation of calcium pectate.

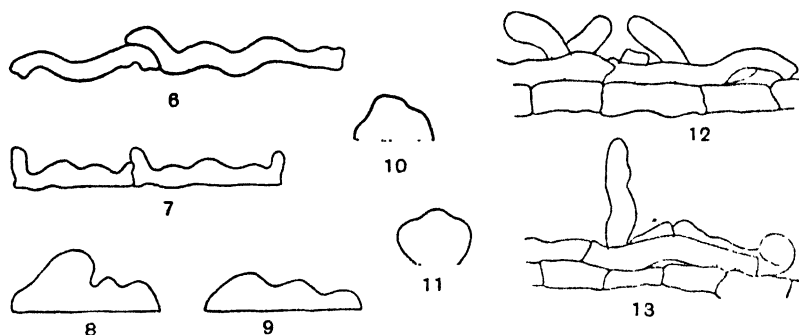
Further experiments were now designed to render unavailable the calcium contained in the root itself. Since ammonium oxalate unites with calcium to form insoluble calcium oxalate crystals, dilute solutions of this salt in distilled water were used. No growth took place in dilute standing solutions. The roots soon became covered with a mucilaginous film containing a large number of minute calcium oxalate crystals.

It was, however, found possible to grow seedlings in dilute flowing solutions of this salt. The rectangular glass dishes in which the corks floated measured 7.5×5 cm. One end of the dish was raised slightly and placed beneath a capillary tube from which the solution dripped slowly. By this arrangement a constant, slow current of solution was kept flowing past the roots, the dish overflowing at the lower end. Seedlings grew slowly in 0.015–0.020 per cent. flowing solutions, attaining a length of about 15 mm. in 2–3 days.

The general effect of the ammonium oxalate solution was to repress root-hair formation. Wide variation occurred due to the individual differences of the seedlings, but with a few exceptions these roots were slender, healthy and white in colour. The arrangement and shape of the epidermal cells were altered to some extent in every

root, but the cell walls were mostly firm and clear. Only occasionally were they covered by a thin film.

The abnormality of the mature tissue was due to the effect of the solution on the cells in the zone of elongation, and upon the long cells in particular. The short cells were least affected, and except in the most severely deformed regions, where they were sometimes slightly swollen or slightly separated, they retained their normal shape and arrangement in even rows. The long cells, on the other hand, were found to be variously separated from each other, and assumed all manner of peculiar shapes, a few of which are illustrated and described in Text-figs. 6-13. The swollen cells often project 40-100 μ



Text-figs. 6-13. Chinese cabbage root; various forms of abnormal long cells produced in ammonium oxalate solutions.

6. The end walls sliding over each other. $\times 90$

7. The end walls elevated vertically. $\times 90$

8-11. Various types of swollen cells. $\times 90$.

12-13. Separation of the long cells indicating lack of stability in the primary wall. $\times 110$.

beyond the common surface of the root, bulging over the short cells. The degree of abnormality varied from root to root, but it was found possible to classify the roots into three different types based on root-hair development.

Type 1. Roots with no distinct hairless region, but covered by a scattering of short hairs, 25-250 μ in length. The condition of the long cells in such plants was variable. They were either flat, slightly swollen and separated, or greatly swollen.

Type 2. Roots with different zones. Hairless regions alternated with regions of papillae and sparse short hairs. The long cells were often greatly swollen and distorted, especially in the regions where

papillae or very short hairs were developed. As a result the root has a distinctly corrugated appearance.

Type 3. Roots that were hairless or practically so from the top to the tip. The epidermal cells were long and flat, slightly swollen and wavy, or extremely swollen and deformed. The root surface was thus either smooth and flat or distinctly corrugated.

In solutions with a greater concentration than 0.020 per cent. of ammonium oxalate, the roots died. It was found possible to keep them alive, however, while producing still greater abnormalities, by the use of composite solutions in which the place of the additional ammonium oxalate was taken by other similar salts. Three such solutions were made with 0.015 per cent. ammonium oxalate solution as a base. In one case 0.25-0.75 c.c. of 1 per cent. potassium tetra-oxalate solution was added per litre, in another 0.25-0.50 c.c. of 1 per cent. oxalic acid, and in a third, 0.25-0.50 c.c. of 1 per cent. citric acid. In all cases similar results were produced. The outline of the root was distinctly wavy or deeply corrugated. The long cells were usually of the long, slightly swollen type, nearly every cell being disconnected from adjoining cells in some manner. Some were free at both ends as in Text-fig. 12 (p. 40), while others were attached only at one end and were either erect, as in Text-fig. 13, or prostrate over adjacent cells. These roots were entirely hairless or produced at most only a very few papillae and short hairs. All of them had a startlingly abnormal appearance, common to them alone, and not observed when roots were grown in any other experimental solution.

The branching of root hairs was found to be a variable feature in flowing ammonium oxalate solutions, with or without the other salts. In some instances many roots were found with either a few or a great number of short, peculiarly branched hairs. At other times none, or only a few roots of this type occurred. When present they resembled those described in the tap-water experiments, and they seem to be connected in all cases with a scarcity but not a complete absence of calcium. Text-fig. 5 (p. 33) illustrates such hairs which were abundantly developed at room temperature on thirteen of eighteen roots of one typical experiment with a flowing 0.015 per cent. ammonium oxalate solution to which five drops of 1 per cent. oxalic acid per litre had been added.

Ammonium citrate, like ammonium oxalate, has the property of removing calcium from solutions of its salts. Great difficulty was experienced in keeping roots alive in ammonium citrate solutions. In flowing solutions of a strength of 0.005-0.007 per cent., however,

some of the roots grew with results similar to those observed in earlier experiments. Hairs were short, though abundantly developed. The swollen long cells were more numerous as a rule. In some roots the swellings projected 70μ out beyond the common surface of the root, bulging laterally over the short cells. In other roots many long cells were bent back and twisted out of position, while some, forced completely out of line, were standing on end. In most hair-producing roots, the hairs extended to very near the tip, probably owing to a reduction in the frequency of cell division. The solution was sufficient to prevent all reaction between the pectic acid and the calcium in the case of the long cells, which after some days presented merely a very thin cellulose wall, covered by a dense film, the pectic nature of which was indicated by deep staining with ruthenium red. It was not sufficient to prevent calcium pectate reaction under the more favourable conditions presented by the short cells, the outer walls of which retained their firm outline.

In the roots produced in ammonium oxalate or ammonium citrate solutions, the test for cellulose was always positive in the walls of both long and short cells, even the most abnormal. More rapid action occurred after heating in 2 per cent. ammonia or in 1 per cent. potassium hydroxide for a few minutes. Calcium pectate was present in the walls of all cells that formed hairs and to a lesser extent in the hairless short cells, but was completely absent in the long cell walls, even in the older regions of the root where it would normally be present.

The importance of calcium to both root and root-hair development was strikingly demonstrated in a series of recovery experiments.

When the deformed roots growing in the various ammonium oxalate solutions measured 15-25 mm. in length, the cork on which they were growing was removed and placed in flowing tap water or in flowing calcium sulphate or calcium phosphate solutions. Immediate recovery characterised by an obvious increase in the diameter of the root and by a sudden burst of long, straight, narrow hairs always occurred. The region of new growth appeared as a distinct bulge, standing in sharp contrast to the narrow deformed region above. There was increased cell division in the meristem, the number of rows of cells being greatly augmented, as well as the number of cells in each row. A sudden increase in horizontal expansion of both the epidermal and cortical cells in the region of elongation was also noticeable. In most roots there was a short interzone of about 100-300 μ , above the bulge. There the short cells, still in the elon-

gating stage at the time of immersion in the calcium solution, remained very short and developed hairs, equal in length to those produced in the enlarged region below. In the long cells lateral swelling and abnormal growth in length was checked, though they remained hairless. In a few roots the response by the short cells was so rapid that several well-developed hairs were formed in the region where swelling of the long cells had not yet ceased. In the enlarged region of new growth, the zone of elongation was greatly reduced, as well as the zone of the young growing hairs.

Table I shows measurements of the length of the epidermal cells in various regions of a recovered root and is typical of the results consistently obtained.

TABLE I

Region of root		Length of long cells in microns	Length of short cells in microns
Ammonium oxalate region	Greatest deformity--hairless	285-520	84-120
	Root diameter 325 μ	Av. 360	Av. 95
Interzone	Appearance of first hairs	120-225	57-75
	Root diameter increasing slightly	Av. 170	Av. 60
Calcium sulphate region	Dense hair development	60-120	24-40
	Root diameter 705 μ	Av. 97	Av. 28

Plate I, fig. 2, shows a section through an exceedingly deformed region of an ammonium oxalate root. The short cells are buried between the long cells which are obviously swollen and misshapen. Plate I, fig. 3, is through the same kind of root after transfer to a solution of calcium sulphate in tap water.

In recovered roots the pectic acid of the cell walls, even in the deformed region, was found to have combined with calcium to form calcium pectate. The same reaction occurred when deformed roots were severed from the plant and soaked in a concentrated solution of calcium hydroxide.

The results of the experiments with oxalate and citrate solutions may be summarised as follows. In roots grown in the absence of sufficient calcium, the root hairs become abnormal or in extreme cases are absent. The abnormalities are always such as can be explained on the basis of internal pressure on a wall that has been unequally hardened. The absence of hairs is accompanied by plasticity of the whole wall of the hair-producing cell allowing expansion in any direction. Efforts to deprive the walls of the short cells of all calcium for the formation of calcium pectate were unsuccessful. In the case of the long cells the complete elimination of calcium pectate in the walls

was brought about, with the result that the cells continued to expand indefinitely. Removal of affected roots to solutions not deficient in available calcium caused immediate recovery of normal growth. It also brought about rapid hardening of the walls of the badly distorted long cells, after which chemical tests indicated that their pectic acid had been transformed into calcium pectate. The whole series of experiments serves to strengthen the hypothesis that root hairs are evaginations produced by internal pressure on plastic portions of an unequally hardened cell wall, and that the hardening is due to the incorporation of calcium in the primary wall.

EFFECTS OF HYDROGEN-ION CONCENTRATION

The long cells fail to incorporate calcium with the pectic acid of their walls and to form root hairs, while the short cells in adjacent rows are able to do so. A provisional hypothesis was conceived that an acid reaction in the long cells might be responsible for the failure of their walls to take up calcium. Pectic acid is a weak acid, practically insoluble in water, and would not be expected to react with the calcium in the presence of a stronger acid. Experimentally it was found impossible to bring about such a reaction *in vitro* in an acid solution.

The cells were therefore tested for hydrogen-ion concentration, neutral red and congo red being used as indicator solutions. Thin strips of epidermis were mounted in the stains and quickly examined under the microscope. The short cells, where they began to develop hairs, had a *pH* value of 5.8-6.8, while that of the growing hairs varied from 6.6 to 7.2. The long cells in the region of elongation and root-hair initiation indicated a value of 4.6-4.8. Further from the tip, where they began the incorporation of calcium into their walls, their *pH* had risen to ± 6.0 . The greater acidity of the long cells was thus clearly demonstrated.

To test the validity of the hypothesis still further, roots were placed in flowing saturated solutions of calcium sulphate made alkaline by an addition of either potassium hydroxide or calcium hydroxide. The effect was greatly to stimulate root-hair production up to an optimum *pH* of 7.8 or slightly higher.

In general roots raised in these solutions were shorter and much thicker than roots grown in tap water or less alkaline solutions. All the cells were extremely short and the proportion of long cell rows was reduced. The important feature is the fact that the long cells

as well as the short cells formed long hairs, making these the most densely haired roots observed during this work.

Individual variations were exhibited by the roots, as was the case with those grown in the ammonium oxalate solutions, and this individuality was, like that shown in the former experiments, based on the greater or less ability to take up calcium and incorporate it in the form of calcium pectate. Here as in the former case there were three types of root.

Type 1. Roots that were stocky and more or less uniform in thickness from the top to the tip. These roots, illustrated in Text-fig. 14, were the most densely haired, both long and short cells producing hairs which measured 2-3 mm. in length. They were considered to correspond to the roots of type 1 growing in ammonium oxalate solutions, which produced a scattering of short hairs.

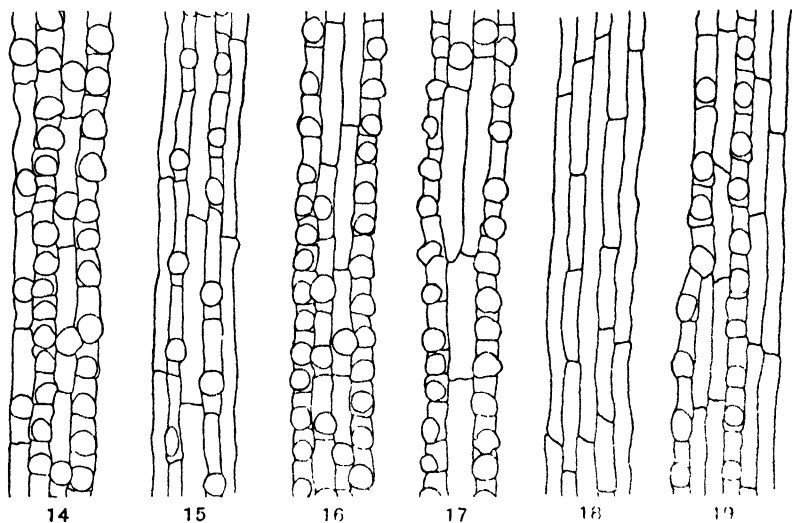
Type 2. Roots with alternating zones of denser and sparser hairs. The densely haired zones resembled parts of the type 1 root, while in the others all the cells were longer and hairs were produced only by the rows of short cells. Sometimes as many as four such zones were seen on a root of approximately 20 mm. in length.

The camera lucida drawings, Text-figs. 15, 16 and 17, show epidermal cells from three successive regions in such a root. The first region of dense hairs in this root is not illustrated.

Type 3. Roots that were densely haired from the top to the tip, but with hairs produced only by the short cells. All the cells were extremely short and the rows of short, hair-producing cells were separated by single long cell rows, a marked contrast to the normal condition, where rows of long hairless cells are produced in much greater proportion. These were the most sparsely haired roots obtained in calcium solutions of the pH mentioned, and so correspond to the type 3 roots in ammonium oxalate solutions.

The cause of the peculiar action of the long cells is thus made clear. In normal conditions the long cells remain acid throughout their period of elongation, and thus either the calcium ions are unable to unite with the pectic acid, or the reaction takes place very slowly, the soft, plastic nature of the middle lamella allowing the cells to elongate rapidly. In a favourable alkaline solution this acidity is overcome. The middle lamella hardens to calcium pectate, and vertical elongation is thus arrested, while the cells are still short. Under these conditions the increasing pressure from within causes a small papilla to be pushed out in the least calcified spot on the cell wall. The papilla develops into a rapidly growing hair.

Additional evidence concerning the above experiments was obtained by the growth of the seedlings in various concentrations of calcium hydroxide in distilled water. In general the development of root hairs was inferior to that in calcium sulphate and calcium phosphate solutions. Here as before it varied widely, depending on the hydrogen-ion concentration as well as the molar concentration of the solution. Many different concentrations were prepared by adding



Text-figs. 14-17 Chinese cabbage root, a few rows of epidermal cells showing the different types of root-hair development in alkaline calcium salt solutions. $\times 110$.

14 Type 1, hairs formed by both long and short cells.

15, 16 and 17 Type 2, successive zones of varying root-hair density on the same root.

Text-figs. 18-19. Chinese cabbage root $\times 110$.

18 Epidermis developed in ammonium oxalate solution and typical of the flat, hairless type of root.

19. Epidermis of the same root after transfer to alkaline ammonium oxalate solution showing decrease in length of all the cells and production of root hairs by the short cells.

various amounts of a saturated solution of calcium hydroxide to distilled water. In some of the most dilute solutions the pH was lowered by adding a few drops of hydrochloric acid. The growth of root hairs in a solution prepared by adding 2 c.c. of saturated calcium hydroxide solution to 1 litre of water with a pH value of 6.0-6.2 was poor. The roots were very slender and showed a hairless region at the top, followed by a scattering of short hairs and papillae, which soon

ceased to form, the roots becoming hairless towards the tip. All the cells were much elongated and the long cells were often slightly swollen. Increasing the concentration and raising the pH to 6.8-7.0 brought about a plentiful development. In most roots the hairs were quite densely developed and were usually long, straight and narrow. Occasionally a few roots produced branched hairs. Some roots showed zonation, short regions of less elongated, hair-producing cells alternating with hairless regions. On increasing the concentration sufficiently to raise the pH to 8.0 a greatly increased development of long, straight, narrow hairs ensued.

Increasing the alkalinity above this point reduced hair production and brought about mild injury to the epidermal cells. In some roots, however, short zones of long hairs were formed towards the tip.

Still more concentrated solutions led to severe injury of epidermis and cortex as well. Farr was the first to discover the rupturing of the cortex of *Georgia collards* in alkaline solutions of calcium chloride and associated it with the cessation of growth in that region, and continued vertical growth of the stelar tissue. In the present investigation it was evident from many observations that the long cells became broken in solutions not sufficiently alkaline to bring about a rupture of the cortex and the short cells. Frequently rows of hairless, almost cubical short cells were found separated from each other by wide jagged gaps. The short cells themselves were generally intact, and full of protoplasm, and only occasionally was the outer wall broken. Apparently the walls of the long cells had hardened to calcium pectate while the cells were still osmotically active, and therefore the outer wall was broken. The short cells on the contrary had not reached the vacuolating stage and so they seldom burst. Whenever hairs developed on these roots, they were either long, straight and narrow, or short and of the "duplex" type. Hairs of this type were found first by Farr, developing most commonly in alkaline solutions of calcium nitrate. In the present work they were found to be produced by the short cells and they were quite uniform in appearance. The base though wide was not swollen, the forking taking place very close to the surface of the superficial cell. The shape of these hairs and their common occurrence in alkaline calcium solutions indicate that the tip of the original papilla had hardened, but that finally the sides yielded and two evaginations grew out instead of one. This is substantiated by the presence on the same root and in the same region of many papillae and very short hairs, the growth of which had been completely arrested by the hardened walls.

In solutions of from 25 to 30 c.c. of saturated calcium hydroxide per litre, severe rupturing of both the epidermal and cortical cells took place. The roots after 3-4 days measured only 10-15 mm. in length, and were entirely hairless, or on an occasional root a few papillae were formed towards the tip. The injury usually stopped above the tip, but in some roots it extended to the root cap, which in such cases was partly separated from the meristem. Wide lateral grooves were produced in most roots, and frequently in several places the root was almost severed in two. In such roots it was almost impossible to distinguish the epidermal cells from the cortical cells. Plate I, fig. 4, illustrates a typically ruptured root. Cell division was stimulated rather than hindered by the increased alkalinity. Occasionally many hairless lateral roots developed, even in the most severely torn regions, where the stele was exposed in wide lateral furrows.

After heating these roots in 2 per cent. ammonia, for $1\frac{1}{2}$ hours, it was impossible to obtain a thin piece of epidermis. Only thick pieces could be broken off, all the cells being firmly cemented together. When the cellulose had been removed by copper oxide ammonia these walls still remained firm. The pectic acid of both cortex and epidermis had been converted into calcium pectate.

When roots grown in tap water for 12-24 hours were transferred to such a concentration of calcium hydroxide, severe rupturing always took place. This was due to the rapid hardening of both epidermal and cortical cells in the comparatively long region of elongation. Conversely, when severely ruptured roots were transferred to tap water with or without additional calcium salts, new vigorous growth was obvious. Hairs were densely developed in the new region, but never in the ruptured region above.

Similar injurious results were brought about by adding small amounts either of calcium hydroxide or of potassium hydroxide respectively to standing tap water. The efficacy of the latter salt indicates the presence of sufficient calcium in untreated tap water, provided the alkalinity is such that rapid reaction with pectic acid occurs. Occasionally the roots, though severely ruptured at first, became more or less adapted to the conditions. In such cases rupturing ceased and frequently a few short hairs developed towards the tip. Adding small amounts of barium hydroxide to tap water had somewhat the same effect, but the roots were unhealthy as compared to those grown in calcium solutions. No growth took place in barium hydroxide solutions in distilled water.

This work is in agreement with that of Farn concerning the

rupturing of the root in alkaline solutions. The observation is added, however, that it is caused by the too-rapid change of the pectic acid lamella of the elongating epidermal and cortical cells to a strongly calcified pectate. Vertical elongation in these cells is thus retarded or completely checked, but it continues for some time in the central cylinder. As a result the rigid walls become torn and broken.

In this experiment an interesting result of the antagonistic action of calcium and potassium ions on the cell protoplasm was observed. With very dilute calcium hydroxide solutions, to which potassium hydroxide had been added, rupturing of the cells took place at a lower pH than in solutions containing more calcium. It is known that the presence of calcium ions tends to decrease the permeability of protoplasm, while potassium ions produce the opposite effect. With the root in a dilute calcium solution made alkaline by potassium hydroxide, we have a very permeable protoplast, which absorbs water with abnormal rapidity and brings about a rupture of the cell walls. In a solution with greater concentration of calcium, the protoplasm is less permeable, and the comparatively slow intake of water does not rupture the walls but stretches them instead.

Another effect of dilute calcium solutions with potassium hydroxide added is that at the optimum pH the hairs, though just as numerous, are not as long as when more calcium is present in the solution. This is doubtless due to the leaching out of osmotically active substances through the permeable cytoplasm, with the result that the osmotic pressure inside the cell rapidly becomes too low to provide for further stretching of the gradually hardening wall.

The marked effect of alkalinity in making calcium more available for the formation of calcium pectate suggested another experiment which further confirmed the results. Seedlings that had been growing in an ammonium oxalate solution that precipitated the calcium, and prevented its reaction with pectic acid, were transferred to a solution of equal strength, to each litre of which had been added in one case 0.5 c.c. and in another 1 c.c. of 1 per cent. potassium hydroxide solution. The deformity ceased, the cells were less elongated, and in many roots a sparse development of short hairs ensued. Chemical tests showed that mature long cells from this region had some calcium pectate in their walls, while in the deformed or hairless region above they were still lacking in this constituent. The potassium hydroxide had made the calcium still free in the seedling more available for cell-wall formation. Text-figs. 18 and 19 (p. 46) illustrate the change which takes place under such conditions.

CONFIRMATORY TESTS WITH OTHER SPECIES

Six species of plants were chosen to be used as tests of the general applicability of the principles suggested by the experiments: Georgia collards (*Brassica oleracea* L.), white mustard (*Brassica alba* L. Boiss.), radish (*Raphanus sativus* L.), endive (*Chicorium Endivia* L.), tomato (*Lycopersicum esculentum* Mill.), Maize (*Zea mays* L.).

The roots of Georgia collards are similar to cabbage roots in appearance and react in a corresponding manner to the various solutions.

White mustard and radish roots are thicker, but they are similar to cabbage roots as regards the arrangement of the epidermal cells into long- and short-cell rows. The cells are wider and there is usually a greater proportion of short-cell rows. They grow poorly in flowing ammonium oxalate solutions. Abnormalities are produced, however, identical with those that arise under similar conditions in Chinese cabbage roots. They respond to slightly alkaline calcium salt solutions in the same manner as cabbage roots; cell division is increased, vertical elongation is arrested and hair development is greatly stimulated. In the most densely haired regions two to three adjacent short-cell rows are formed, while the long cells may or may not develop long hairs. In the concentrated calcium hydroxide solutions, the roots are very thick and hairless, and the cortex is ruptured.

Endive and tomato roots show no arrangement of the epidermal cells into long- and short-cell rows. The cells vary in length over different regions of the root and any cell may form a hair. Hairs are, however, sparsely developed in tap water. They are greatly stimulated in alkaline calcium salt solutions, every cell forming a hair in most roots. There is a clear relationship between the length of the cell and the formation of root hairs. For example, on tomato roots growing in tap water, hairs were sparsely developed and the cells measured 150–300 μ longitudinally, while in alkaline calcium salt solutions they measured 60–150 μ , and every cell formed a long hair. A common feature of tomato roots is the mingling of long hairs and papillae. Miss Roberts reported that this was a frequent occurrence when roots of alfalfa, cabbage and *Verbascum* were grown in moist air.

The epidermal cells of maize roots are not differentiated. In air every cell forms a hair, while in water the roots are typically hairless. Since the available calcium was as great in water as in air, it was suggested that the failure to form hairs in water might be due to an

acid reaction. This suggestion was strengthened by the fact that in calcium sulphate solutions hairs were only erratically developed or were not produced at all. To test this theory small amounts of potassium hydroxide solution were added to tap water and to dilute calcium salt solutions in tap water. By this method hairs were produced. The optimum production was when 200 c.c. of saturated solution of calcium sulphate were added per litre of tap water, and the pH regulated between 7.2 and 8.2. The maize seedlings were placed on corks floating in one quart glass sealers. These were placed in large glass jars immersed in the constant temperature tanks at temperatures of 15 and 18° C. and were illuminated by artificial light. After 2-3 days the roots measured 3-5 cm. in length and they were either straight or spirally coiled. All roots were not alike, but, in general, root hairs were densely developed, and some roots were just as densely haired as those growing in moist air. In such roots the cells were shorter than the hairless epidermal cells developed in tap water alone, and every cell formed a hair. The hairs were straight and narrow and measured from papillae to 500 μ in length. Through such treatment the acidity of the epidermal cells, due, doubtless, to anaerobic respiration, was neutralised and the calcium ions were thus made available to unite with pectic acid. Calcium pectate was formed and normal root-hair production resulted.

CONCLUSIONS

The wall of a normal root hair has a cellulose and a pectic layer, continuous with corresponding layers in the epidermal cell. In all the species investigated tests for other wall substances were negative, though the occurrence of "protopectin" in the wall of the adult hair is possible. While the hair is growing the cellulose layer is soft and easily hydrolysed and gives every indication of being the same over the whole surface. The pectic layer on the sides of the hair is of firm calcium pectate, and the same is true of the parent cell walls with the exception, sometimes, of that on the inner side of the cell. On the dome-shaped hair tip this layer is composed either of pectic acid or of a softer calcium pectate. In the mature hair the cellulose wall is less easily hydrolysed, and the whole surface is covered by a layer of calcium pectate of the firmer type.

When the amount of calcium available for wall formation is reduced, other factors remaining constant, the hairs are abnormal, sometimes inflated as though the side walls were too soft to withstand the internal pressure, and often branched as if the pressure had

distended certain spots softer than the rest of the wall. With further reduction in the amount of calcium, there is no formation of hairs, and the cells expand more or less symmetrically. Roots failed to grow in solutions capable of wholly excluding calcium from the walls of those cells that would normally have produced hairs.

The explanation of the plasticity of walls only partially deprived of calcium was found when the reaction between calcium and pectic acid was investigated *in vitro*. When the pectic acid was treated with a very dilute neutral or slightly alkaline solution of a calcium salt, a substance was precipitated that reacted to solubility tests as calcium pectate, but was softer than the calcium pectate produced when a concentrated solution was used.

In at least half of the species investigated there were rows of "long cells" with a metabolism different from that of the hair-producing cells. In the region of hair formation, the pectic layer of their walls did not react with calcium, and they produced no hairs. These cells were found to be considerably more acid than the hair-forming cells at this stage. At a later stage they became less acid and the pectic acid layer of their walls changed to calcium pectate. In solutions deprived of calcium they retained the pectic acid layer indefinitely, and became greatly elongated, distorted and swollen. Experiments proved that pectic acid and calcium fail to unite in an acid solution, thus providing the clue to this behaviour.

When roots were allowed to grow in a solution with sufficient calcium and with a pH of 7.2-8.2 the long cells, with their acidity neutralised, produced normal hairs. In still more alkaline solutions the walls hardened so rapidly that no hairs at all were produced, the walls were broken by the expanding contents, and in extreme cases epidermal and cortical cells were pulled apart leaving deep clefts.

Some species produce roots that when growing in water or in nutrient solutions containing calcium are hairless or practically so, although in air they have an abundance of hairs. *Zea mays* is an extreme example. The failure to produce hairs is apparently due to the production of acid by the roots when air is excluded, for in alkaline calcium solutions hairs were produced abundantly.

The experiments indicate that two conditions must be fulfilled if normal root hairs are to be produced. In the first place a supply of calcium in the form of a dissolved calcium salt must be present. Secondly, the hydrogen-ion concentration must be such that a reaction between the calcium salt and the pectic acid of the cell wall

may take place *gradually*. Through the gradual hardening of the cell wall vertical elongation is arrested and, due to increasing pressure from within, the wall is pushed out at its softest point, invariably at or near the distal end of the cell. The progressive hardening of the outer layer confines this softer spot to a narrow area at the growing tip where new wall is being constantly laid down, and this determines the narrow diameter of the full-grown hair.

This problem was undertaken at the suggestion of Prof. H. B. Sifton, to whom I am greatly indebted for advice and constant assistance throughout the investigation. I also wish to thank Dr D. H. Hamly for his friendly help with the photomicrographs.

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EXPLANATION OF PLATE I

- Fig. 1. Chinese cabbage root, transverse section through the normal meristematic region. $\times 127$.
- Fig. 2. Chinese cabbage root, transverse section through the abnormal region developed in ammonium oxalate solution. $\times 127$ (s.c., short cells).
- Fig. 3. Chinese cabbage root; transverse section through the recovered region developed after transfer to a solution of calcium sulphate in tap water. $\times 127$. Hair bases indicate the short cells.
- Fig. 4. Chinese cabbage root; cortex ruptured in a strongly alkaline calcium hydroxide solution. $\times 7$.

Fig. 1

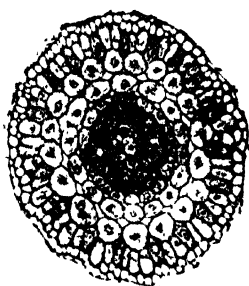


Fig. 2

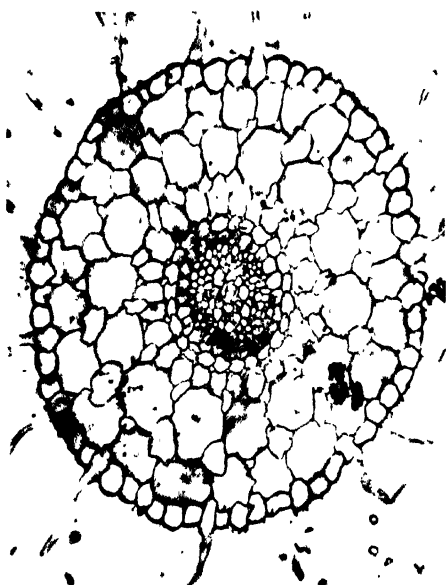
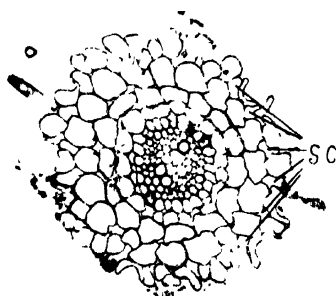


Fig. 3



Fig. 4

NON-SYMBIOTIC DEVELOPMENT OF SEED- LINGS OF *EPACRIS IMPRESSA* LABILL.

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(With Plate II and 4 figures in the text)

I. INTRODUCTION

A CONSIDERABLE amount of work has been done on the non-symbiotic development of the seedlings of *Calluna vulgaris* and other members of the Ericaceae with somewhat conflicting results. On the one hand, Rayner(5-10) claimed that for normal seedling development it is necessary to establish infection of the seedlings by the fungus which is constantly associated with the plant growing under natural conditions and which may form a mycorrhizal complex of a rather special type. A fungus, which has been repeatedly isolated by Rayner from the fruit capsules and seeds and named *Phoma radici-Callunae*, is regarded as the fungal component of the association, and for normal root development to take place she considers that it is necessary for this fungus to be brought into contact with the young seedling at an early stage of its history.

Christoph(2), on the other hand, obtained satisfactory germination and, under most conditions, normal seedling development of this plant when the seeds were sown aseptically without the presence of the fungus. Christoph recorded that when he placed unsterilised seeds of *Calluna* on peat which had been sterilised by repeated autoclaving or on sterilised soil, he produced seedlings with a normal rooting system which in all cases were entirely free from fungus infection. Similarly, sterilised seeds on sterilised soil gave well-rooted seedlings which at five months were also entirely free from hyphae, whereas in control experiments on unsterilised peat or soil he produced seedlings the roots of which showed the typical mycorrhizal association.

Rayner(6) considered that all seedlings raised from unsterilised seed should "be assumed liable to infection at germination" and suggested that the presence of mycelium in the roots of plants raised in this way had been overlooked by Christoph. Rayner stressed that even when a typical mycorrhizal association may be absent, root

infection may still be present but in such an attenuated form that it might readily not be recognised. In commenting on the results obtained when sterilised seeds were employed, Rayner considered more adequate proof of seed sterility was necessary. It might be pointed out here, however, that Christoph used 1 per cent. mercuric chloride as the sterilising agent and Rayner(9) contended that this was the most efficient antiseptic that she had been able to find for seed coat sterilisation of *Calluna* and *Vaccinium* seeds. Christoph did not maintain his cultures under sterile conditions throughout the duration of the experiment, so that subsequent air-borne infection of the substrata with the appropriate fungus was not excluded.

"Pure culture" work is undoubtedly necessary before results should be accepted without reserve.

Knudson(3) also obtained excellent germination and normal seedling development when he grew seeds on Rayner's solution A, solidified with 1.5 per cent. agar; to some of the culture tubes 2 per cent. glucose was added and calcium hypochlorite was the sterilising agent employed. He attributed the abnormal development noted in Rayner's seedlings to be due to a toxic effect, which he considered might be operating in her experiments, and which might offer the explanation for the non-development of the root system under such conditions. He suggested that excess of iron in the culture solution might to some extent be responsible for the toxic action, but as Rayner recorded lack of root development even when the seeds were sown in water on sterile filter paper, Knudson realised that this explanation was not entirely adequate.

The toxic action of mercuric chloride used in seed sterilisation he thought to be a more probable factor in producing abnormal seedlings, but this hypothesis, as pointed out by him, does not account for the normal development of seedlings in the presence of the fungus, as recorded by Rayner from seeds treated in exactly the same way as those which produced abnormal seedlings.

Rayner(10) in reply criticised Knudson's experiments, particularly the calcium hypochlorite method of seed sterilisation. After trial she found it unsatisfactory for *Calluna* and other ericaceous seeds and considered it inadequate to destroy the mycelium of the endophytic fungus which she claimed to be present on the seed testas. Root development in Knudson's seedlings could, according to Rayner, be explained on the basis of incomplete sterilisation and the consequent infection of the seedling at germination. The reported absence of fungal hyphae in such roots is discussed and the difficulty of demon-

strating the presence of hyphae in them is again offered as an explanation of the discordant character of Knudson's and her own results. Rayner considered hyphae are present in the seedling roots but they have been overlooked. Knudson(4) met this criticism of his methods by reinvestigating the problem and he sets out the procedure adopted by him for seed sterilisation, cultural work, and staining methods in great detail. He again found that *Calluna* seeds germinated well on a mineral salts medium, with or without the addition of sugar, and in both series after some considerable interval of time the roots of seedlings were well developed and showed no sign of fungal infection. In contrast, seeds sown on potato-dextrose agar were found to produce seedlings with abnormal root development. As these resembled the asymbiotic seedlings described by Rayner, Knudson thought that the peptone-dextrose medium used by her, as well as his potato-dextrose agar, was toxic to the seedlings or "lacking in some essential nutrient, the deficiency of which prevents root-growth." To test this idea he sowed *Calluna* seed on peptone-dextrose agar. In cultures in which 1 per cent. peptone and 1 per cent. dextrose were present roots failed to develop, whereas when the amount was reduced to 0.1 per cent. in each case, normally rooted seedlings resulted. Knudson concluded that peptone is toxic and, in his experiments, was an inhibiting factor to root development; other substances can apparently act in the same way, for potato-dextrose agar also gave stunted roots. Summing up, he states, "with a favourable culture medium with or without soluble organic matter seedlings in my experiments develop with healthy normal roots and without the aid of the fungus. Seedling development of *Calluna vulgaris* therefore does not require the aid of *Phoma radidis Callunae*."

II. MYCORRHIZA IN THE EPACRIDACEAE

The family Epacridaceae is practically confined to the Australian-New Zealand region and takes the place in the Australian flora that is occupied by the Ericaceae in the European zone. The two families are closely related, the chief morphological characters which separate them being centred in the stamens. It has long been thought that Epacridaceous plants would exhibit specialised relations with root fungi, but as pointed out by Rayner(6) no investigational work of this nature has been recorded on plants belonging to this family. In an unpublished thesis Baron(1) working with *Epacris impressa* Labill. reported the presence in its roots of a mycorrhizal fungus.

The general histological characters of the smaller roots and the details of the mycorrhizal structure agreed closely with those already described for *Calluna vulgaris* (4). Root hairs are absent and hyphae invest the younger parts of the root abundantly, penetrating in numerous places into the outer cells, either by passing directly through the cell wall or occasionally by pushing in between two adjacent cells. Distortion of the hypha was often noticeable at the point of penetration. The outer root cells were filled with densely branched hyphal complexes and all stages in digestion of these masses were observed. As the Epacridaceae is closely allied to the Ericaceae and the mycorrhiza in the roots is identical, Baron and the writer carried out a critical examination of the shoot tissues of *Epacris impressa* including the floral organs and seed capsules with the object of demonstrating the presence of hyphae in the aerial parts of this plant, as has been recorded by Rayner for *Calluna*. The results were entirely negative, although extensive search was made, using material fixed and stained according to recognised cytological methods.

III. SEEDLING DEVELOPMENT

Parallel with the histological investigation germination of the seed of *Epacris impressa* and subsequent development of the seedling under pure culture conditions were undertaken primarily with the idea of procuring further evidence of the distribution of the mycorrhizal hyphae in the tissues of this plant.

Unfortunately the work was interrupted at an early stage, but some of the cultures set up by Baron were retained and others were commenced later by the writer. The latter have been under observation now for three years (1930-3) and the following facts are thought worthy of record.

The seeds are very small and light and under laboratory conditions do not germinate readily. Various treatments were accorded the seed samples, the most favourable results following after exposure to ether vapour in a desiccator (Rayner⁽⁹⁾). After various time periods had been tried, the seeds were finally subjected to the action of the vapour for one and a half hours. Sterilisation with the calcium hypochlorite method followed; this method has been found eminently satisfactory in this laboratory for use with many kinds of seeds. The seeds were shaken in the solution for about three-quarters of an hour, to ensure thorough wetting. They were then sown (a) directly on to the surface of sterile nutrient agar jelly in Erlenmeyer flasks, the flasks being kept after germination at laboratory temperatures

or in a cool glass house, or (b) on to the surface of sterile sand moistened with a similar nutrient medium as that employed in making up the agar cultures without the addition of the agar. The mineral salt medium chosen was Rayner's solution A:

Potassium nitrate (KNO_3)	1.0	gram.
Magnesium sulphate (MgSO_4)	0.4	"
Calcium sulphate (CaSO_4)	0.5	"
Calcium monophosphate ($\text{CaH}_4\text{P}_2\text{O}_8$)	0.5	"
Sodium chloride (NaCl)	0.5	"
Ferric chloride (FeCl_3)		Trace
Water	2000	c.c.

To this was added varying proportions of agar from 1 to 1.5 per cent. and dextrose in 2 per cent. concentration. The hydrogen-ion concentrations were varied over a range of 4.5-5.2, but no one concentration proved more favourable for growth and in later experiments the pH 4.8 was used. The seeds were sown on to a horizontal agar or sand surface in 300 c.c. flasks and incubated at 22° C.

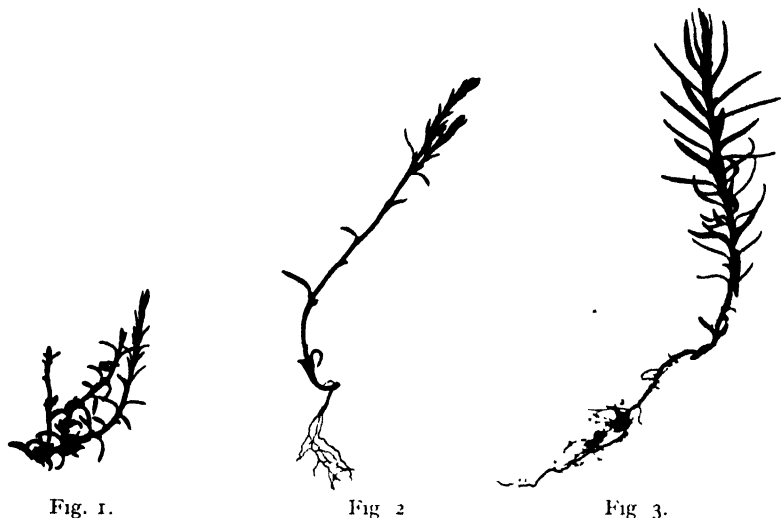
Germination of the seeds was observed eight weeks after sowing. After three months the seedlings in the agar flasks had developed an aerial shoot on an average about 2-3 cm. long bearing minute foliage leaves, but there was no development of a normal root system. The hypocotyl region in practically all cases curved upwards and was abruptly terminated by a root tip around which were developed rudiments of lateral roots, invisible to the naked eye. Some of these seedlings were later transferred to sterilised sand cultures moistened with Rayner's solution plus dextrose.

The seedlings in the agar series continued to develop green aerial shoots and although not so large as control seedlings grown in unsterilised soil, nevertheless appeared normal and continued to grow and develop aerial shoots in spite of the non-development of a root system. Some of the seedlings at the end of one year were removed from the culture flasks for photographic record.

Text-fig. 1 represents the plantlets in the agar series; there is no development at all of a rooting system, yet there was evidently sufficient absorption taking place in the hypocotyl region for the plant to continue to grow and to develop healthy green shoots. Text-fig. 2 represents the plantlets in the sand series, both those developed from seeds sown directly on to sand as well as those that had been transferred from the agar flasks to sand after three months. At transference these seedlings showed the hypocotyl ending abruptly in a root tip without any formation of true roots. The root system

of all seedlings in this series was fairly developed at the time of photographing and in striking contrast to those of the agar series. Text-fig. 3 represents control seedlings grown in unsterilised soil and raised from seed treated in the same way as in the other series; that is, the seed was both etherised and sterilised before sowing.

From time to time many of the agar seedlings were transferred to larger flasks containing nutrient agar and their development was followed for three years. In some cases extensive branching of the aerial system occurred, resulting in a tufted plant, while in other



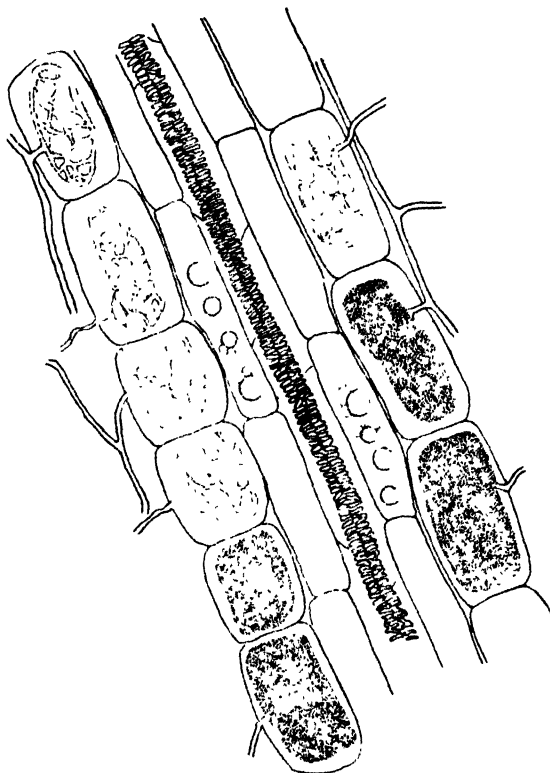
- Fig. 1. Seedling of *Epacris impressa*. 1 year old. Grown on nutrient agar, note absence of visible roots.
 Text-fig. 2. Seedling of same age, grown on sand moistened with nutrient solution, note root development.
 Text-fig. 3. Seedling of same age, grown on unsterilised soil.

flasks this typical healthy character was not so noticeable (see Plate II, figs. 1 and 2).

In all cases the shoots appeared a normal green colour and the plants were vigorous and healthy. At the end of three years the flasks were dismantled and a thorough examination was made of the bases of these plants to see if any trace of fungal mycelium was present in the tissues. After fixation in Flemming's fixative, microtomed sections were stained with Haidenhein's iron-alum haematoxylin, Flemming's triple and Breinl's stain, but in no case could any fungal infection be detected.

The plants from sterilised sand moistened with the nutrient solu-

tion and dextrose were kept under aseptic conditions for eighteen months when the flasks were dismantled and these plants were examined. The striking difference between the sand plants and the agar plants was the development of a normal fibrous root system in the former. The roots were subjected to a critical examination both before and after fixation. Sections were stained with a number of



Text-fig. 4 Diagram of a rootlet of *Epacris impressa* to show the distribution of the mycorrhiza in the root cells.

recognised cytological stains, and in addition intact roots were cleared and mounted in lacto-phenol after staining with cotton blue dissolved in lacto-phenol solution; but again no trace of hyphae in or between the root cells or in the stem and leaf tissues could be detected.

Parallel with these experiments *Epacris impressa* seedlings grown in unsterilised soil were observed and they were found to develop root systems which to the eye resembled those formed by the seedlings grown in the sterilised sand series. These roots, however, on

sectioning showed heavy infection with a mycorrhizal fungus, the cytological detail of which is similar to that recorded for *Calluna* by Rayner(7) (Text-fig. 4).

IV. DISCUSSION

As the Ericaceae and Epacridaceae are closely related and placed, as well as some other families, by Engler in the Ericales, the results obtained by germinating *Epacris impressa* seeds aseptically on nutrient agar jelly and sterilised sand moistened with a nutrient medium are of interest in connection with the controversy which has taken place over seedling development in *Calluna vulgaris* under similar conditions.

Although seedlings were raised on nutrient agar which failed to develop roots, such plants were grown on agar in the laboratory for three years and although somewhat stunted they nevertheless developed vigorously and many aerial shoots were formed despite the fact that no real root system was ever realised.

The non-development of a typical root system in *Epacris impressa* when seedlings are grown in Rayner's solution in agar plus the addition of sugar is not due to either:

- (a) the absence of the mycorrhizal fungus, or
- (b) to the agent used for seed sterilisation,

for seeds treated at the same time and in the same way as those sown on nutrient agar but placed on sterilised sand moistened with Rayner's solution A + sugar developed normal root systems which, however, showed no sign of fungal infection. It seems quite clear that root development in this form is not dependent on infection by the mycorrhizal fungus.

The factor which varied in the experiments was the actual rooting medium, the agar in the flasks presented a horizontal surface to the germinating seeds and evidently the gel was such that the fine rootlets of *Epacris* either could not penetrate into the medium, or their development was arrested physically or chemically by the gel, so that the primary root tip remained capping the hypocotyl region. In sand no such arresting action was exercised, so that penetration of the rooting medium by the fine rootlets was possible, the root rudiments developed and a root system was established.

The development or non-development of roots in these experiments seems to rest on the physical or chemical character of the rooting medium, and is not obligate to infection of the young seedling by its appropriate fungus.

The production of seedlings, free from any fungus infection, from



Fig. 2



Fig. 1

sterilised seeds grown aseptically, is evidence that the distribution of the mycorrhiza in the Epacridaceae is not similar to that described for the Ericaceae by Rayner. This evidence is supported by the failure to find any hyphae in the aerial tissues of the normal plants.

V. SUMMARY

1. The presence of a mycorrhiza in the roots of the Epacridaceae similar to that in the roots of Ericaceae is recorded.

2. Examination of the aerial tissues has not revealed any trace of fungal infection in contrast to the Ericaceae.

3. *Epacris impressa* seedlings were raised asymbiotically on nutrient agar gels and on sterile sand moistened with a nutrient solution.

4. Plantlets on agar failed to develop roots but were nevertheless grown in the laboratory for three years and healthy green shoots characterised these plants.

5. Plantlets on sand, plus a nutrient solution, were normal in their development but free from mycorrhizal infection.

6. Plantlets grown from sterilised seed on ordinary unsterilised soil were normal in their development and had a typical root mycorrhiza.

7. The physical character of the rooting medium seemed to inhibit root formation and its absence is in no way the result of non-infection by the appropriate mycorrhizal form.

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EXPLANATION OF PLATE II

Fig. 1. Seedlings in flask growing on agar gel. 2½ years old. Note tufted healthy character of growth.

Fig. 2. Seedlings in a similar flask showing a more straggling habit.

OBSERVATIONS ON *FUSICLADIUM* *SALICIPERDUM*

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(With 1 figure in the text)

A DISEASE of willows (especially *Salix fragilis* var. *decipiens*), associated with the fungus *Fusicladium saliciperdatum* (Allesch. and Tub.) Tub., has been prevalent around Cambridge during recent years. First the leaves and then the young twigs become black, and in a wet season the trees may be almost wholly defoliated before midsummer. During dry weather, however, the progress of the disease is completely stopped. Cankers may be formed on the stems where the disease is checked. Around Cambridge, pustules of *Fusicladium saliciperdatum* invariably develop on the leaves and twigs affected in this manner, the twigs becoming invaded later by a variety of secondary organisms. Records of the occurrence of *F. saliciperdatum* in other parts of England are given in Bulletin No. 79 of the Ministry of Agriculture (*Fungus and other Diseases of Crops*, 1928-32).

This leaf and twig blight of willows has often been described in Europe, notably by Tubeuf(1), Schwarz(2) and Alcock(3), and in the United States by Clinton and McCormick(4). On the ground of successful inoculations the latter authors claim that *F. saliciperdatum* is the cause of this disease. On the other hand, Nattrass(5) and Dennis(6) failed to infect willow leaves with this fungus, whereas they readily induced infection with *Physalospora Miyabeana* Fukushi, which causes a disease practically indistinguishable as regards symptoms from that associated with *Fusicladium saliciperdatum*. They inclined to the view that the latter was a secondary organism having no pronounced parasitic tendencies.

In view of the discrepancy between the results of Nattrass and Dennis and those of Clinton and McCormick it was decided to examine further this disease of willows as it occurs around Cambridge, especially as *Physalospora Miyabeana* was never seen on the affected material.

The characters of *Fusicladium saliciperdatum* have been sufficiently described by the authors cited above. It is easy to establish pure

cultures of it on the usual media, but in our experience the cultures soon cease to produce spores except on malt agar. There is ample evidence that the fungus overwinters in the twigs killed in the previous season, and we can confirm the presence of sporing pustules on these twigs just before the leaves become affected in the spring.

If *F. saliciperdu* is indeed responsible for the willow disease described above, there are some features about its pathogenicity which are markedly different from the pathological symptoms usually induced by other species of *Fusicladium*. Such species do not generally kill outright the leaves and twigs which they invade, whereas *F. saliciperdu* is associated with the rapid destruction of the tissues permeated by it. During the hot summers of 1933 and 1934 we have occasionally seen parts of leaves, attacked on the upper surface by *F. dendriticum*, *F. pirinum* or *F. Pyracanthae*, which have been killed down to the lower surface, thereby producing fairly large brown spots. This effect, however, is hardly comparable with the rapid extension of the destructive action associated with *F. saliciperdu*.

In May 1932 some trees of *Salix alba* var. *vitellina* growing in the open and young plants of the same variety established in pots were inoculated with spores of *Fusicladium saliciperdu*. The results were inconclusive, as only one of several trees inoculated in the open was infected and of the pot plants one alone became doubtfully infected. Inoculations of cut twigs and detached leaves of *Salix fragilis* var. *decipiens* kept under moist conditions failed to give rise to the disease.

In May 1934 opportunity was afforded to carry out inoculations under better conditions. Young plants of *Salix fragilis* var. *decipiens* had been previously established in pots from sets kindly sent from the willow collection at Long Ashton by Mr H. P. Hutchinson. On 17 May several uninjured shoots of each of three plants were inoculated with spores of *Fusicladium saliciperdu* applied by means of a brush: (a) to the upper surface of fully developed leaves, (b) to the under surface of similar leaves, and (c) to buds just beginning to proliferate. The plants were covered with bell-jars throughout the duration of the experiment. The first sign of infection was seen on 28 May, when blackish blotches began to appear on various parts of the inoculated leaves, precisely similar to those characteristic of the disease in nature. In extending over the leaves the blackening followed particularly the line of the midrib towards the petiole, which also soon became affected. In some instances the discoloration spread from the base of the petiole into the young stem, again reproducing one of the symptoms of the disease in nature. Occasionally an

incipient canker was formed at the downward limit of the blackened part of the stem. Pustules of *F. saliciperdatum* developed abundantly on the diseased leaves, especially along the lines of the veins on the lower surface. All the inoculated shoots became infected, so that the fungus can invade both the upper and the under surface of the leaves. The control shoots remained healthy. It is clear, therefore, that *F. saliciperdatum* may behave as a true parasite and that it is capable of causing a serious disease of willows under certain conditions.

There is one remarkable feature about the pathogenicity of *F. saliciperdatum* as seen in the above experiments. Once the blackening of infected leaves has begun the discoloration extends with great rapidity, certainly faster than can be accounted for by growth of the fungus in the tissues. The blackening extends outwards from one or more centres in the leaf. Fig. 1 indicates the rate of extension of the discoloration of the leaf lamina.

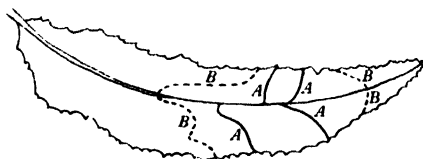


Fig. 1. At 2 p.m. on 28 May the leaf appeared normal. At 4 p.m. the part of the lamina bounded by the continuous line A was black. At 7 p.m. the blackening had extended to the broken line B.

It would appear that, once the host cells have begun to be killed by the fungus, neighbouring cells are rapidly killed in like manner in ever widening zones until the entire leaf may become involved. Perhaps some product of the first cells to be killed has a lethal effect upon contiguous cells, this effect being reproduced continuously. The blackening of the tissues is doubtless due to an oxidase reaction. Another possible explanation of the rapid extension of blackening is that a toxic secretion of the fungus itself, rather than any substance produced by the dead host cells, is generally distributed in the infected leaf, and that the concentration of this toxic secretion increases outwards from certain foci of lethal strength as time elapses so that the tissues are killed progressively. Whatever be the explanation of the rapid extension of this discoloration, it is noteworthy that if, for example, the apical part of a leaf is cut off so as to remove the whole of the black tissues, the blackening again begins to develop near the cut edge of the remainder of the leaf within 12 hours. That the dis-

coloration is due to the intervention of the fungus follows from the fact that uninoculated leaves, parts of which have been cut off or injured by burning, do not show any extension of necrosis beyond the regions directly killed by such treatment.

From the results of these investigations and from other researches it seems certain that *Fusicladium saliciperdatum* and *Physalospora Miyabeana* can both cause serious diseases of young willow shoots, the symptoms of which are practically indistinguishable from each other. With regard to the distribution of the two diseases in this country, observations in the west of England (Long Ashton) indicate that *P. Miyabeana* is almost always, perhaps invariably, the cause of the blackening of willow leaves and young stems, while around Cambridge *Fusicladium saliciperdatum* in our experience has constantly been responsible for this affection.

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REVIEWS

The Gramineae. A Study of Cereal, Bamboo and Grass. By AGNES ARBER, M.A., D.Sc. 6.4×9.3 in. Pp. 480, coloured frontispiece and 212 text-figures. Cambridge University Press, 1934. Price 30s.

In 1881, George Bentham, in an address on the Gramineae to the Fellows of the Linnean Society of London, said: "The paramount importance of the Gramineae in an economical point of view has called forth innumerable treatises, memoirs and essays on cereals, on forage and other cultivated grasses, on meadows and pastures, on ornamental grasses, on the physiology and properties of the Order, etc." During the supervening fifty-three years considerable additional work has been done, and a further copious literature has resulted, adding studies in embryo development, seedling morphology and anatomy, floral morphology, genetics, and ecology. Mrs Arber's contribution to the more recent of this literature is already widely known, and its value in the elucidation of morphological problems fully appreciated.

The present volume is the fruit of some 30 years of consideration of grass problems either as such or in their relation to the larger field of general morphology. Mrs Arber's grass researches had their origin in her close association with Ethel Sargent, to whom she gracefully acknowledges indebtedness for training in the methods of research, traced further to the influence of D. H. Scott, to whom the volume is dedicated.

The subject-matter is arranged in an interesting and orderly way in spite of the difficult nature of the task. Dealing, as it does, with a large mass of accumulated research, much of which is original, all is welded together in a masterly way and presented with great clarity, every point supported with a wealth of illustrations taken from early systematic literature, or, more largely, from the author's own researches. The first few chapters are a review of the cereals of the Old and New Worlds, their origins hidden in remote antiquity, their culture from the dawn of history, their distribution as spreading out from their respective centres, and the races into which they have become subdivided, so that their field has become the world, and their culture finds employment for the races of mankind. Next come the pasture grasses, and others which are of use to man, such as the sugar cane. Whilst passing mention is made of grasses which furnish scents, no reference is made to those which are utilised in medicine, possibly because they are so few and relatively unimportant, though the medicinal virtues of at least one grass have given it pharmacopoeial status, namely couch-grass rhizome, and three others have been officially recognised as the source of amylin.

A consideration of the bamboos occupies four succeeding chapters, and through their study we are introduced to the principles of the morphology of the graminaceous flower. We are even taken into the realms of speculation as to whether, supposing the Angiosperms to be monophyletic, the primeval ancestor was arboreal or herbaceous, and as to the significance of woodiness and the tree habit generally. Whilst the author puts forward a forceful argument in support of primitive herbaceousness, weaknesses in it are evident, such as when the bamboos are compared with the woody Dicotyledons, which is quite unjustifiable on anatomical grounds alone, and when woodiness in general is described as a kind of pathological state denoting senility.

Under the description of the reproductive shoot we have introduced the behaviour of its parts at anthesis, and certain phenomena are ascribed to

turgour, viz. (1) the movements of the inflorescence branches by the pulvini at their bases, (2) the swelling of the lodicules causing the opening of the flower, (3) the extension of the stamen filaments. No experimental evidence is adduced for (1); it is one of a number of common phenomena, such as the movements of the involucre bracts of the capitula of Compositae about whose mechanism we have, rather ashamedly, to confess our ignorance.

The process of reduction, by which the typical grass flower is evolved from that represented by the bamboo, is related to the effects of overcrowding and compression due to ontogeny. The author also detects an inherent tendency towards some degree of sterilisation of the reproductive shoot in the grasses. Her views on the morphology of the grass embryo and seedling are put forward. Following this the vegetative phase is next considered, and the old morphological categories come under review. Mrs Arber recognises in the "root" and "shoot" a fundamental correspondence which places them in primary and equivalent categories, and, thinking back to origins, accepts as a provisional working hypothesis the Lignier theory that there is no fundamental difference between stem and leaf.

Under the heading of distribution and dispersal is made the somewhat misleading statement: "Various grasses, again, are so constituted that they can endure strongly saline water, and hence are able to grow by the seaside in salt-marshes or on sand-hills. Of these *Psamma arenaria*, *Elymus arenarius* and *Glyceria maritima* are familiar on our shores." Actually only the last of the three mentioned comes directly under the influence of strongly saline water, by being submerged in it at times. The other two are usually outside the zone of direct sea influence. Several other grasses share with *Glyceria maritima* the power to survive immersion in salt water.

Natural hybridisation amongst the grasses is considerable, both between species of the same genus, and to some extent between different genera. The histories of maize and of Townsend's cord grass are of some interest in this connection.

Lastly, with the taxonomy of the grasses, a suggestion is made that the name Gramineae should be used co-equally with Glumiflorae as an order, and that the family name should be Gramineaceae, comparable with Cyperaceae. At the last meeting of the International Botanical Congress at Cambridge in 1930 the name Gramineae was recognised as the family name and placed with others not ending in -aceae, but familiarised by common usage, under *nomen conservanda*. The rule would have to be revised in accordance with Mrs Arber's suggestion before the latter could be brought into use.

Proliferation of spikelets is a fairly common occurrence amongst grasses: the term "vivipary," though generally understood, is certainly misapplied here. The condition can be induced in grasses under cultivation, and the causal circumstances are clearly different in different species. General statements such as "examples are easiest found in autumn, especially after a wet summer; excess of water is a predisposing cause" are not always applicable, as in the case of "viviparous" forms of *Festuca ovina* examined by Jenkin, who found that the condition is inherent, and the production of proliferated spikelets takes place early in the year, with some formation of normal spikelets later.

The book is fully indexed and is printed in clear type on good paper. It is in keeping with the traditions of the Cambridge University Press. One spelling mistake appears to have slipped notice in the final revision of proofs: on page 89, line 9, the context suggests that "brake" is meant for "break." All who are interested in grass problems, from whatever angle, will find in this volume a useful work of reference, and those who are interested in morphological problems generally will, one hopes, be provoked to attempt, by further research, to fill in the gaps in our knowledge which are clearly indicated throughout the book.

Practical Plant Anatomy. By C. J. A. BERKELEY. $7\frac{1}{4} \times 4\frac{3}{4}$ in. Pp. 112, with 19 figures in the text. University of London Press, London, 1934. 3s.

This little book is written both for students taking the London Intermediate Examination and for their teachers.

It describes what a microscope is and how it should be used and how to cut sections. It then goes on to describe nearly a hundred anatomical "exercises"—chiefly the study of sections (including microchemical reactions) of stems, roots and leaves of flowering plants. Finally, there is some information for teachers about botanical materials.

The style throughout is clear, and though concise, explicit enough for students who have the help of demonstrators. The directions given are sound, but I dislike some of the conventions suggested for drawing cells. The ground covered may be rather more than most students would master, but a teacher could omit exercises or could use the book after the intermediate stage.

There is one bad feature, which may sound trivial, but will probably prevent many teachers from putting this book into a student's hands: this fault is the very small scale of the drawings of cells. In the drawing of the tissues of a sunflower stem several hundred tiny cells are represented—in one place about a hundred in less than 2 sq. cm. This, it is true, agrees with the figures in the standard text-books, but in such a book of practical instruction it is essential that the figures should be thoroughly good because a student is sure to take them as a model. Rather ironically the text around the figure impresses the fact that cells should always be drawn large but are often represented too small. I doubt if a student could draw from a section three or four hundred cells representing them individually and accurately in less than four hours, and I am sure no one could do it on such a small scale. I know that I would soon be found covering the paper with neat little hexagons designed to give a general impression of the tissues—a thing which a student who is anything of a draughtsman quickly learns to do, unless checked, with diabolic skill and to the admiration of his fellows. It is a pity such a figure, which embodies some excellent features, was not simpler and printed at several times this scale, because this and similar slight alterations would have made the book a thoroughly good guide for students.

As it is, this book has many excellent features which will make it very useful indeed to school teachers, who often find themselves cut off from the elementary practical botany of Universities, and no doubt most university teachers will be able to pick up useful suggestions, but I cannot recommend it unreservedly for students.

T. M. HARRIS.

Researches on Fungi. Vol. VI. By A. H. R. BULLER. 9.4×6.2 in. Pp. xii+513, with 231 figures. Longmans, Green and Co., London, 1934. 28s. net.

With the publication of his sixth volume of *Researches* Prof. Buller adds still further to the story of spore dispersal in the fungi, the subject which has always formed the central theme of his work. He presents us with pictures and descriptions which bring before our minds images of solid living organisms giving a pleasing sense of reality. Any one interested in the fungi cannot fail to read this work with considerable enjoyment.

The book is divided into three parts. Part I deals exhaustively with the biology of *Pilobolus*. The first chapter is an excellent summary of the history of our knowledge of this fungus and will be welcomed not only by mycologists

but also by physiologists interested in phototropism and exudation. The second chapter deals most minutely with the structure of the *Pilobolus* gun and the discharge of the sporangium. Buller regards the subsporangial bulb as the lens of a simple eye focusing the light on the reddish orange zone of protoplasm which forms a perforated septum at the base of the bulb. This protoplasm he regards as analogous to the retina of the human eye. Convincing diagrams show how the lens focuses light on the retinal zone, and experiments are described in which this was observed in the living organism. It is suggested that when this zone is not evenly illuminated curvature occurs just below the orange red region until the "retina" is symmetrically illuminated. In the diagrams constructed to show the paths of the rays of light through the lens, Buller omits to take into account the exuded drops on the surface of the bulb which, acting as minute lenses, might cast disturbing bright spots on the protoplasm. Although it would be a very pretty arrangement if the subsporangial bulb and the orange red protoplasm at its base could be shown to constitute an eye, nevertheless it seems clear that no direct evidence is presented to support the theory. Against the "eye" theory it may be mentioned that the young sporangiothere is phototropic before the subsporangial bulb is formed, and that in the allied genus *Phycomyces* a similar phototropic response occurs although there is no suggestion of an "eye" mechanism to perceive the light.

The account of the actual discharge of the sporangium is a beautiful piece of work. A very probable theory is advanced of how it comes about that the discharged sporangium sticks to any object which it strikes so that the spores are covered by the black cap of the sporangium.

An explanation is given of the curious fact, first noticed by Allen and Jolivette, that when a culture of *Pilobolus* is confronted with two equal sources of light, curvature of an individual is towards one or the other without any sporangiothere taking up an intermediate position. The explanation agrees with that put forward by Van de Wey but was reached independently.

Chapter III is largely devoted to the description of a new species of *Pilobolus*, *P. umbonatus*. This very distinct new form was discovered by Hans Ritter, aged eleven.

The final chapter of Part I is by W. B. Grove who contributes a systematic account of the Pilobolidae, including a key to the species of *Pilobolus*. This revision of his earlier monograph of the Pilobolidae, published fifty years ago, will remain for a long time the standard systematic treatment of these fungi. One small but interesting feature of this chapter is a reproduction of the first known drawing of *Pilobolus* published in 1691.

In Part II Prof. Buller returns to the subject of spore discharge in the Discomycetes which occupied part of his first volume of *Researches*. He makes some important contributions to our knowledge of the phenomenon of "puffing." He shows conclusively that heliotropism of the asci is not a peculiarity of the genus *Ascobolus* and its near allies but is general throughout the Discomycetes. This prevents wastage of spores in species which have the form of a hollow cone such as *Sarcoscypha protracta* or which have a highly contorted hymenium with resulting alveoli as in *Morchella*. Particularly interesting is the account of *Sarcoscypha protracta* in which only the very tip of the ascus is heliotropic, so that no general curvature of the ascus results. In *Aleuria vesiculosa* not only the asci but also the paraphyses are positively heliotropic. Much of Buller's earlier success in studying the Hymenomycetes resulted from his looking at the living hymenium in surface view under the microscope. He has now applied this method with considerable success in the case of Discomycetes.

Clear evidence is presented that puffing sets in motion the whole body of air immediately above an apothecium, the upward draught produced assisting in the dispersal of the spores.

A light-hearted chapter is devoted to the sounds made by fungus guns. We are told that, on puffing, the apothecium of a Discomycete makes a noise

like a rush of steam from a minute jet. Perhaps some day the puffing *Discomycete* will, like the nightingale, find a place in the programmes of the B.B.C.

In the first two chapters of Part III the "rooting base" or pseudorhiza of certain toadstools is considered and is shown to connect the fruit body with the mycelium vegetating in some such buried substratum as a woody root. In most cases the pseudorhiza is annual, but in *Collybia fusipes* a perennial pseudorhiza is formed, resembling in function the rhizome of higher plants. In the cup fungus *Sarcoscypha protracta* a very similar perennial pseudorhiza is found.

The last chapter is mainly devoted to the peculiar fungus *Omphalia flavida*, a luminous agaric responsible for the American coffee-leaf disease. Normal sporophores are produced and also much smaller gemmifers. These are without doubt specialised sporophores in which lamellae and basidia are not produced, but the whole pileus becomes detached from its stipe and forms a vegetative reproductive unit or gemma. The account of *Omphalia flavida* is one of the most interesting parts of the book.

As in previous volumes a general summary at the end of the book contains a précis of each chapter, and reference to the text is made easy by a most comprehensive index.

Although the book is very readable and well produced it is much too long and would have gained in value by being condensed to half its present size. Many of the illustrations, though pleasing, are quite unnecessary and could have been omitted with advantage. Prof. Buller would render a great service to students of botany if he would produce a concise book of about 250 pages summarising all his researches on spore discharge in the fungi and illustrated by a careful selection of his own beautiful figures.

C. T. INGOLD.

Die Buchenwälder Europas. Heft 8. Edited by E. RÜBEL. Veröffentlichungen des Geobotanischen Institutes Rübel in Zürich. Pp. 512. Huber, Bern und Berlin, 1932. RM. 20.80.

At a meeting of the International Phytogeographic Excursion in 1923 a scheme was agreed upon for the unification of ecological research by a combined attack throughout Europe upon the problems presented by the native beech forests. Seven years later at the International Congress in Cambridge European ecologists presented their results in a symposium, arranged by Prof. Tansley, and now we have to thank Prof. Rübel for the publication in book form of the products of these co-ordinated labours. And our thanks should indeed be sincere, for this work represents a very great advance in our knowledge of European forests and indicates the enormous advantages of co-ordinated research along phytogeographic and phytosociological lines, as well perhaps as showing some of the practical difficulties of such co-ordination. Certainly no botanical or forestry department in Europe should fail to consult the book.

The content of rich ecological material is so great that the reviewer can only pick out for comment one feature of particular interest, namely the true outlines of the distribution of *Fagus sylvatica* as a forest dominant in Europe, and the causes suggested as determining the limits of this distribution in different directions.

The vertical distribution is described by Rübel as a layer 1000 m thick over the greater part of Europe, with gaps and irregularities: from north to south the layer gets higher above sea-level and also becomes thinner. In the north only an upper limit is recognisable, and in the south only a lower. The upper limits are shown by the series: Southern England, 300 m.; Hartz Mountains, 470 m.; Riesengebirge, 950 m.; Bayrischer Wald, 1000 m.; Northern Alps, 1200 m.; Jura, 1300 (locally 1570) m.; Tessin, 1800 m.; Etna (Sicily), 1900 m.

In the continental east the upper limit is set by the top of the fog belt. Above the beech is wood dominated by *Picea excelsa* or *Larix europea*, and below it is mixed oak forest.

The lateral distribution of beech forests shows its northern limits in Southern Sweden, and Dr Lindquist suggests that the limitation operates partly by summer temperatures being too low to ripen mast and to initiate flower production. In addition he considers that late spring frosts, by freezing the stigmas of the flowers, very strongly affect the mast crop. The north-western limit is partly set by the soil-climate interaction which causes podsolisation and raw-humus soils on which beech regenerates much less readily than on the mull soils. The north-eastern limit is related to a low rainfall (500 mm.) operating especially through spring droughts. In Germany the eastern limit of the beech is more readily recognisable than the western, it passes through Pomerania, and is considered by Markgraf to operate by low winter temperatures, late frosts and a rainfall below 500 mm. Southwards this continental limit passes into Poland, and Szafer attributes the limitation to late spring frosts, to a rainfall below 550 mm. and to sudden falls of temperature in summer. The last factor has a great influence in the plains and restricts the beech to the hills, though the rainfall factor often operates similarly. In the Balkans, Rumania and the Crimea the position is complicated by the occurrence of *Fagus orientalis*, a species of different ecological requirements which penetrates farther east than *F. sylvatica*. Variation in both species and hybridisation between them make it difficult to draw exactly the south-eastern limit of the European beech. *F. sylvatica* reaches its southern limit in mid-Greece. It occurs between 1700 and 2000 m., its lower limits fixed by summer-air dryness which often limits the woods to north-facing slopes, and its upper limits by low winter temperatures or by the top of the fog belt. The beech woods are here near to their eastern limit; the zone being forced higher up the mountains, the extent of the beech area being thus dependent on the presence of sufficiently high mountains. Data are not plentiful along the Mediterranean limit of the beech forests except again in Spain, where the southern limit is very clear. Control appears to operate through summer drought despite high total rainfall (c. 800 mm.). The bulk of the Iberian beech forests are in the Pyrenees, and the islands of beech forests farther south are areas with local climates moister than that of the surrounding country, where the dry summers exclude deciduous trees and the climax vegetation is *Quercetum ilicis*. In the western regions of high rainfall, as in England, the beech appears to prefer calcareous to siliceous soils. In France the beech woods lie above those of fir (*Abies alba*) which appears to withstand the conditions of high rainfall, high humidity, soil acidification and freezing, and particularly wind-action, less well than the beech. Thus in the absence of spruce which reaches its oceanic limit in the Vosges, the beech forms the highest tree zone, and at 1400 m. grows as knee-high scrub very heavily wind-trimmed. The north-western limit of the beech woods passes through the middle of England, and we may invoke to explain it the factors also suggested by Lindquist, namely low summer temperatures, late spring frosts or climatic soil degeneration. The historical factor may possibly also be involved, since it is possible, though unlikely, that the beech is a recent introduction which has not yet reached the final limits of natural distribution in this country.

This short summary deals with one aspect only of the ecology of the beech forests and neglects all the countries in the centre of the distribution area. If it is recalled that each of the sixteen contributors also discusses fully the effects of altitude, topography, soil, human activity, climate and history on the beech forests, and analyses the major plant communities within them, something will be realised of the extensiveness and scope of the work. It would, we venture to think, have been an advantage had the dynamic aspects of the beech forests been as thoroughly worked out for the continental countries as for England by Watt and Tansley, and it is evident that a great deal of the

work is still uncorrelated. Nevertheless the enterprise marks a very real step forward in ecological method, and it is to be hoped that we shall find it applied to other species of general European interest.

H. GODWIN.

Le Haut-Jura neuchâtois nord-occidental. Beiträge zur Geobot. Landesaufnahme der Schweiz. Heft 17. By H. SPINNER. Pp. 197, six plates and two maps. Huber, Bern und Berlin, 1933. RM. 9.60.

This volume deals with the vegetation of those valleys in the Jura on the north-west boundary of Switzerland, the whole territory lying between the extreme altitudes of 915 and 1334 m. The extensive woods of the area consist of spruce, fir and beech, with spruce decidedly the most abundant and beech the least abundant of these three dominant species. The beech has suffered much in the past by cutting for fuel (especially for lime-kilns) and by pasturing, though there has been a reaction in recent times in favour of the beech as a forest tree ameliorating soil conditions. There is an interesting discussion of large experimental plantations of native and American trees and records are given of the extensive damage done by squirrels and by winter snows. Lists of phanerogams are given for the forest regions, with frequency, abundance and life form, and full lists of bryophytes and lichens are also given. Even the "mixed" forests of the area contain little more than 50 per cent. of deciduous trees, chiefly beech, but it is these that first recolonise the juniper scrub on abandoned pastures, and doubtless these would also be more important to-day were it not for the greater commercial value of the conifers and their encouragement by man.

About 20 per cent. of the area is occupied by forest and 40 per cent. by "près bois," different types of wooded pastures which in the absence of grazing rapidly revert to forest. The pastures also include reclaimed peat bogs. Aquatic and marsh vegetation is represented in the Lac des Tailières where the waters have a very high calcium content. The lake has recently been converted into a large reservoir, but a description and map are given of the vegetation of the former lake and its plant communities.

Especially in the valley de la Brévine there are large areas of sphagnum moor, which show all stages of exploitation by cutting and drainage, and of recovery from these processes. The climatic type is considered to include both Hochmoor in the narrow sense of Osvald, and also Waldhochmoor dominated by *Pinus montana*.

Other vegetation types considered are those of bare rocks and of cultivated fields. There are also chapters on phenology and on the history of the vegetation. The latter is much strengthened by a series of pollen analyses from the peat deposits of the area. They show clearly that deciduous trees formerly played a much greater part than now in the forest composition, especially the lime, pollen of which sometimes reached 18 per cent. of the total tree pollen. It appears that in a humid Atlantic period oak and hornbeam were killed out by the fir, spruce and beech and have not been able to reinstate themselves in subsequent periods. The early Atlantic period was marked by a very strong preponderance of the fir pollen, which only in the uppermost peat layers is exceeded in amount by spruce pollen.

The memoir is accompanied by two maps—one showing the former vegetation of the Lac des Tailières, and the other a coloured vegetation map of the whole area on a scale of 1/25,000. The latter, which is an extremely satisfactory and useful contribution to the primary survey of Swiss vegetation, is published by the Commission phytogéographique de la Société Helvétique des Sciences Naturelles.

H. GODWIN.

Flora von Graubünden. Zweite Lieferung. Von J. BRAUN-BLANQUET und E. RÜBEL. Heft 7. Veröffentlichungen des Geobotanischen Institutes Rübel in Zürich. Pp. 435. Huber, Bern und Berlin, 1933. RM. 18.

This volume is a continuation of the very valuable flora of the Graubünden, of which Part I, the *Monocotyledons*, was reviewed in Vol. xxxii of this *Journal*. This part includes the *Dicotyledons* from the *Salicaceae* to the end of the *Rosaceae*. It may be repeated that for each species there is given not only a comprehensive list of occurrences in the different floral regions of the Graubünden, with accompanying altitudes, but also concise and extremely useful ecological notes. The ecologist, as distinct from the systematist or phytogeographer, would desire to see these notes as much enlarged as possible. As it is the reader is often left with a dogmatic statement where he would have preferred a reasoned comment. This is so, for instance, in regard to the vexed question of perglacial survival versus recent spread. Thus the discontinuous distribution of *Ranunculus lingua* is explained as due to transport by water birds, whilst that of *R. pygmaeus* is explained in terms of perglacial survival. The survival hypothesis is also invoked to explain the distribution of *Anemone pulsatilla*, and one would be glad to know in some detail the reasons for choosing one hypothesis and not the other.

As is always noticeable in floras of this type, there are many instances of the change in edaphic requirements of a species in different parts of its geographical range. Thus *Alnus glutinosa* is given for the Graubünden as characteristic of sites by standing water low in calcium content, being replaced by *A. incana* on the basic soils of the river-alluvia. In the east of England, of course, *A. glutinosa* is extremely abundant on alkaline peats and on basic alluvial soils.

This volume is of particular interest because it deals with many of the trees, poplars and salices, alders and birches, oaks, beech and hop-hornbeam. It is remarkable to an ecologist familiar with *Quercus robur* and *Q. sessiliflora* only in English woods to find how sharply separated are their habitats in this part of Switzerland. The former is extremely scattered and occurs in rainy and damp regions, whereas the latter occurs in dry valleys where it becomes a forest dominant. This is almost the opposite of what one would guess from the distribution of the two species in Britain, where *Q. sessiliflora* appears to have a marked preference for the valleys of the west. The accumulation of data of this kind is of importance in many ways. It is, for instance, a necessary key to interpretation of problems of plant geography and the history of the major vegetational units.

H. GODWIN.

Le Valentinis Méridional. Esquisse phytosociologique. By G. DE BANNES-PUYGIRON. Trav. de l'Inst. de Bot. de l'Univ. de Montpellier. Extrait des communications de la Station Internationale de Géobotanique Méditerranéenne et Alpine. Pp. 200. Mari-Lavit, Montpellier, 1933.

Le Valentinis Méridional is a small area of striking topographic diversity which lies in the western part of the Dauphiné, immediately west of the Rhône and south of its tributary, the Drôme. The climax vegetation is divisible into two altitudinal stages, that of *Quercetum pubescentis* from the valley floors to 1000 m., and that of *Fagetum sylvaticae* from 1000 to 1600 m. Both stages of the climax have been subject to the most extreme effects of human interference; the oak forests have been almost completely destroyed and only a

few scattered examples of the beech high forest remain in the most distant and unpopulated parts of the region. It follows that the phytosociological outline given by M. Bannes-Puygiron is very largely concerned with the wide variety of communities produced by different intensities and types of human interference and destruction, and of recovery from these effects. It is evident that here, as in England, intensive field ecology in the vast majority of cases leads inevitably into a background of forest or agricultural history. The *Q. pubescens* woods have suffered by cutting for fuel and for pasturing, and by pollarding for sheets for cattle feeding. Both felling and pasturing favour the growth of the box which is a constant component of the oak woods. In this way secondary box woods arise, though, especially in calcareous regions, primary box woods may arise by direct colonisation of scree. The box is so resistant to destruction, and is so untouched by cattle that with juniper it is often the only tree in over-pastured areas. Less severe destruction of the oak woods gives coppices dominated by hazel and containing the residual woodland species. In many places excessive pasturing after clearing has led to erosion of the soil and to extreme denudation, and a very widespread result of this is the community dominated by *Lavandula vera*. This community is strictly limited to dry calcareous soils and the lavender is locally exploited for scent making. *Lavandula latifolia* which characterises a similar community is of less commercial value. These degradation phases show very high percentages of chamaephytes (50 per cent.) and of therophytes, thereby contrasting strongly with the original climax forest with phanerophytes and hemicryptophytes (50 per cent.) predominant. They also include a large proportion of spiny and strongly smelling plants highly resistant to grazing: the lavenders themselves are of the second class. In addition to these vegetational types due to human activity there are also within the oak stage well-marked communities determined edaphically, such as the valley *Alneta* and communities on calcareous rocks.

Within the beech stage similar series of communities are recognisable. Where local topography produces a climate with very high humidity the fir, *Abies alba*, occurs freely mixed with the beech, and local conditions are also responsible for the very irregular junction between the oak and beech stages, and for the distribution of the woods of *Pinus sylvestris* on warm valley slopes. The communities induced by destruction of the beech forest include not only "Lavandae" and "Buxae" but heaths dominated by chamaephytic calcifuges such as *Calluna vulgaris*, *Genista pilosa*, *G. cinerea*, *Vaccinium myrtillus*. These may even occur in a climatically determined surface soil above a purely calcareous rock. Above the beech forests are communities of herbaceous plants with subalpine species and these penetrate in small patches far down into the beech woods.

The communities are all classified and described according to the principles of M. Braun-Blanquet. The description is interesting throughout but would have been greatly helped by the provision of topographical and geological maps, and possibly also by photographs of the chief communities.

H. GODWIN.

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SEASONAL CHANGES IN ACIDITY OF THE RHUBARB. (*RHEUM HYBRIDUM*)

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(With 4 figures in the text)

INTRODUCTION

MALIC acid occurs in many plants and probably, if sufficiently reliable methods were available for its certain recognition, traces at least would be found in almost all plants. Very great concentrations are found in many plants of which the common rhubarb is one. It has recently been claimed by Ruhland and Wetzel (5, 6) that the malic acid in this plant originates from protein sources and that it is formed together with ammonia by the deamination of amino acids and oxidation of the carbon residues. If this be so, its mode of origin is strikingly different from that of the malic acid produced by succulent plants in which the acid is produced from carbohydrate during the processes of respiration. We have therefore re-examined certain features of the acid metabolism of the rhubarb, and at the outset may point out that our results do not in any way support the hypothesis that the acid originates from protein sources or that its production is associated with an abnormally active protein metabolism; they indicate rather that malic acid production is a process concomitant with the carbohydrate metabolism of the plant.

This reinvestigation seems all the more desirable in that the analytical methods used by the Leipzig school are of doubtful validity and accuracy. Details of these methods have never been published, but the published results show in many cases that the methods are by no means free from objection.

These results (7) imply that the quantities of both "free acid" and "bound acid" are determined analytically. This is not actually possible, though at least two methods are available which yield

results that can readily be confused with the "free acid" and "bound acid" contents of the tissue. If the tissue be dried, free acid can be extracted from the residue with ether; alternatively, free acid can be removed from this residue by sublimation *in vacuo*. When conditions are carefully controlled almost all the free acid in the residue can be removed and estimated by micro-titration or gravimetric methods. But the quantity of free acid in the dry residue is greater than the quantity of free acid in the original solution, since the position of equilibrium between the acid and its ions changes during the process of concentration of the solution in such a way that the degree of dissociation decreases with increasing concentration of the solution. The analytical results so obtained consequently have little or no interest or significance, since the free acid content of the residue bears no simple relationship to the free acid content of the original solution.

The equilibria occurring in solutions of dibasic acids and their salts have already been discussed by one of us(2), and it is only necessary to point out here that the most useful analytical data regarding such solutions in plants are the titratable acidity and total acidity. It is perhaps advisable to define these terms clearly. In the case of an acid H_2M , capable of yielding the ions HM^- and M^{--} on dissociation:

$$\begin{aligned} \text{Total acid} &= [H_2M] + [HM^-] + [M^{--}]. \\ \text{Free undissociated acid} &= [H_2M]. \\ \text{Total free acid} &= [H_2M] + [H^+]. \\ \text{Titratable acid} &= [H_2M] + [H^+] + \frac{1}{2} [HM^-]. \\ \text{Non-titratable acid} &= \text{Total acid} - \text{titratable acid}. \\ &= [B^+] \text{ (i.e. cations other than } H^+). \end{aligned}$$

It should be pointed out that in the case of a weak acid the term $[H^+]$ in the expressions above may be negligibly small, and can ordinarily be neglected. It is not clear whether the expression "free acid" used by the Leipzig school refers to the total free acid or free undissociated acid, but in either case the quantity can only be arrived at by calculation when the pH of the solution is known, and in the case of plant extracts a number of simplifying assumptions have to be made which are only partially true.

The doubtful validity of the Leipzig analytical methods is also clearly shown by some of the published data, which in certain cases do not appear to be in conformity with physico-chemical laws: for example, Schwarze(7) gives the following figures:

TABLE I

Cc. molar acid per 1 gm. dry weight in leaves of Pelargonium

Free acid	... 0.270	Bound <i>l</i> -malic acid	... 0.243
Free <i>l</i> -malic acid	... 0.220	Bound <i>dl</i> -malic acid	... 0.847
Bound acid	... 1.880	Bound oxalic acid	... 0.419
		Bound "rest" acid	... 0.291

The ratio of free *l*-malic/free *d*-malic acid is therefore larger than 9/1 and the ratio of bound *l*-malic/bound *d*-malic acid is only 1.6/1. Since the dissociation constants of optical isomers are equal, the ratios of concentrations of the isomers in the free and bound states must be equal. The very large divergence indicated by the figures quoted may be caused by some analytical error. The possibility is not lost sight of that the living protoplast might maintain the composition of the mixture of the two acids and their salts in a position different from the equilibrium position. But the analyses are of necessity carried out on a non-living solution, and it is almost inconceivable that such a solution should not be at equilibrium since ionic reactions are almost instantaneous.

It would be of great interest to know how these abnormal equilibria are explained by Schwarze, and also to have details of the analytical methods which are claimed to show their existence.

EXPERIMENTAL

(1) *Analytical methods*

The soluble acids and mineral or other salts were extracted from the minced tissue by boiling with water. Aliquot parts of the solution so obtained were analysed. Analytical results are throughout expressed as mg. equivalents per 100 gm. fresh weight of tissue unless stated to the contrary.

(a) *Titratable acidity* was determined by titration with 0.02 N CO₂-free soda to the end-point of *pH* 7.0 using brom-thymol blue as indicator. In general the extract was diluted to avoid interference with the end-point by colour changes due to anthraquinone or anthocyan pigments.

(b) *Total malic acid* determination presents many difficulties, to which it may not be out of place to refer briefly. The malic acid (along with other acids) may be extracted from the dried tissue acidified with phosphoric acid by ether, or it may be sublimed out of the tissue under reduced pressure. Alternatively, it may be precipitated from

the water extract as an insoluble salt. The accuracy of all methods depends then on the completeness with which these processes can be carried out. We have not been able to obtain complete quantitative extraction of the malic acid with ether, and in this our experience agrees with that of Needham(4). The vacuum sublimation technique is under investigation here now. Finally numerous precipitation methods have been proposed. The most widely used methods are those in which the acid is precipitated as calcium, barium, or silver salts from alcoholic solution. We find again that when known small additions of malate are made to plant saps that it is in many cases impossible to recover the whole of the added malate in the form of these salts. This incomplete precipitation of malates is almost certainly due to the protective action of some of the colloidal substances in the extract. The method which we find most reliable is the lead precipitation method developed previously by one of us (Ph.D. thesis, Cambridge University).

The extract from about 1 gm. fresh weight of tissue is exactly neutralised to pH 7, a drop of capryl alcohol is added, and 20 per cent. basic lead acetate is then added drop by drop until no further precipitate forms. In general about 0.1-0.2 c.c. are required: it is particularly important to avoid an excess of lead acetate, as the lead malate goes into colloidal solution in basic lead acetate solution. The precipitation of malate is complete, since the lead acetate also precipitates the substances which prevent complete precipitation in the case of the calcium and barium salts. The precipitate is centrifuged out, washed, decomposed with H_2S , the lead sulphide centrifuged out, and the supernatant liquid titrated with CO_2 -free soda to pH 7.0.

This titration gives the quantity (in mg. equivalents) of the lead-insoluble salts, i.e. malate + citrate + succinate + oxalate + sulphate + phosphate among the more important. The neutralised liquid can then be used for the separate estimation of several of these by standard methods, oxalate as calcium salt, phosphate as uranyl salt, citrate as pentabromacetone, etc., the malate being obtained by difference. Actually in the present investigation malate + citrate + succinate + fumarate (if any) have not been separately estimated in the routine analyses, since the quantities of the last three were believed to be very small, and in any case these acids are all closely related chemically. The combined analytical result is referred to as "malic acid" in this paper. When this paper was first written we thought, following Ruhland and Wetzel's statement, that all or almost all of the optically inactive acid was malic acid, but more

recent work in this laboratory shows that citric acid is also present. Since, however, we do not know how much of the optically inactive acid is citric acid at all seasons we are retaining the terms "malate" and "malic acid". This procedure is at present justifiable, since malic and citric acids are very closely related chemically, and we already regard these two acids and also fumaric succinic and tartaric acids as members of the malic acid group. The term "malate", then (in inverted commas), refers to total acid of the malic acid group. Where the acid is specifically known to be *l*-, *d*- or *i*-malic it will be referred with the necessary prefix.

(c) *Ammonia* was estimated by the van Slyke method, *amides* by the Sacchse method and total nitrogen by the Kjeldahl method.

(d) *Carbohydrates* have been estimated by the copper reduction (cuprous titration) method of Shaffer and Hartmann.

(e) The extent of recovery of malic acid and ammonia from saps, to which known additions had been made, by the use of various analytical techniques is indicated by the results of a few typical experiments recorded in Table II.

TABLE II

Results of some test analyses of sap with known additions of malate and ammonia

Quantity added	Quantity recovered	Method of analysis
<i>i</i> -malic acid		
67.0 mg.	38.6 mg.	Precipitation calcium <i>i</i> -malate
16.8	12.3	
16.80	16.80	Precipitation lead <i>i</i> -malate
3.25	3.30	without excess lead acetate
16.80	9.10	Precipitation lead <i>i</i> -malate
4.00	3.47	with excess of 5 times
		correct amount lead acetate
16.80	14.20	Ether extraction
Ammonium chloride		van Slyke method
4.15 mg. N	2.27 mg. N	15 min. bubbling
4.15	4.15	45 min. bubbling
4.15	4.15	1 hour 45 min. bubbling

(f) The variety of rhubarb used was "Daw's Champion".

(ii) *Changes in composition during sprouting*

A number of preliminary experiments were carried out in which pieces of rhizome bearing a bud were allowed to sprout in darkness at 23° C. One of these will be dealt with in detail. The rhizomes in the open ground in January are in the resting condition. One of these

was brought indoors, washed free of earth and divided into two equal parts each of which weighed 276 gm. One part was analysed at once, and the other placed in darkness at 23° C. The leaves commenced to elongate rapidly after about a week and were over 6 in. long after 15 days; at this stage the second half-rhizome and the leaves which had sprouted from it were analysed.

The results of these analyses are expressed as quantities per whole plant. We assume that the two half-rhizomes are identical in composition at the start of the experiment, and other experiments indicate that errors of the order of magnitude of about 15 per cent. are involved in this assumption. The analytical results are given in some detail in Table III.

TABLE III

*Quantities of various substances in the half-plants
D₁ and D₂ before and after sprouting*

	D ₁ , from open ground Jan 16th, whole plant	D ₂ , after 15 days' growth at 23° C.			Change in whole plant %
		Rhizome	Leaves	Whole plant	
Fresh weight	276	255	44	299	+ 8.3
Ammonium N, mg. equivalents	4.90	0.72	1.32	2.04	- 58.2
Amide N x 2, mg. equivalents	19.2	13.5	0.0	13.5	- 29.6
Rest N, mg. equivalents	45.6	32.5	3.9	36.4	- 20.2
Insoluble N, mg. equivalents	28.2	29.0	0.6	29.6	+ 5.0
Total N, mg. equivalents	97.9	75.7	5.8	81.5	- 16.9
Glucose, gm.	0.65	0.46	0.05	0.51	- 20.2
Sucrose, gm.	7.70	1.55	0.08	1.63	- 78.8
Polysaccharide hydrolysable by 1 % HCl, gm.	1.74	0	0	0	- 100.0
Total carbohydrate, gm.	10.10	2.01	0.13	2.14	- 78.6
Total acid, mg. equivalents	41.1	33.2	11.8	45.0	+ 9.8
Mg. carbon as acid*	980	797	283	1080	+ 9.8
Mg. carbon as carbohydrate (excluding "skeletal" carbohydrate)	4040	804	52	855	- 78.6
Mg. carbon as protein + amino acid + amide†	4480	3600	215	3800	- 15.0

* Assuming malic or citric acid.

† Assuming that 1 atom of nitrogen is associated with 4 atoms of carbon.

It will be noted that the process of sprouting is associated with small increases in fresh weight, acid content, and insoluble nitrogen (largely protein), and with decreases in soluble nitrogen including ammonia, and very large decrease in carbohydrate. The fact that total nitrogen decreases some 17 per cent. is ascribable to sampling

error, as it is improbable that much change in this could take place. Bearing this in mind, the extents of the changes in quantity of various nitrogenous compounds are not very considerable: these changes are in the expected direction, new growth being associated with increase in quantity of protein at the expense of soluble nitrogen compounds. The very large loss of carbohydrate is associated with the abnormally high rate of CO_2 output when rhubarb buds sprout at temperatures as high as 23°C . The slight increase in quantity of "malic acid" is of doubtful significance, and there is no reason whatever to suppose that this acid has arisen as a by-product of deamination of amino acids, since in actual fact the quantity of ammonium nitrogen decreases rather markedly.

We have considered the actual quantities of the various substances present. The concentrations can be worked out from the figures given, and it at once appears that the young petiole has a lower amino acid ("rest" N) concentration than the resting rhizome and higher total "malate" and ammonium concentrations. So far as we can see, these *concentration* relationships must be the source of Ruhland and Wetzel's(5) conclusion that malic acid and ammonia are produced by "active deamination" of amino acids in the petioles. When the actual *quantities* of the products are considered this hypothesis is seen to have no foundation in fact. The concentration relations just referred to are evidently conditioned by the translocation mechanisms of the plant, or perhaps it would be more strictly accurate to state that they can be explained more readily by supposing them to be due to translocation effects rather than to metabolic changes involving deamination.

Other experiments of this type have yielded essentially similar results.

(iii) *Seasonal march in total "malate" content of
different parts of the plant*

A series of experiments was carried out to determine whether rhubarb plants contain more "malate" than the piece of rhizome out of which they develop, and at what season the increase in acid content (if any) occurs. To attain this object it is necessary to refer the acid content of a plant of any given age not to the fresh weight of the plant, but to the original acid content of the piece of rhizome out of which the plant grew. This, in practice, is most readily done by referring analytical results to the original fresh weight of the piece of resting rhizome which gave rise to the plant. The legitimacy of this

operation depends of course on the degree of similarity of the original pieces of rhizome. The total "malate" determinations are accordingly recorded in the first place as quantities (mg. equivalents) per 100 gm. fresh weight of the original piece of resting rhizome (this is abbreviated to "per 100 gm. O.F.W.").

About a dozen pieces of rhizome each bearing buds were cleaned free from soil and weighed and planted on February 19th. One small "crown" from each was taken and analysed in its original state, and the plants which developed from the remainder were analysed from time to time during the season. The quantities of total acid, *l*-malate, oxalate, and ammonium were determined and also the titratable acidity. The first of these is dealt with in this section.

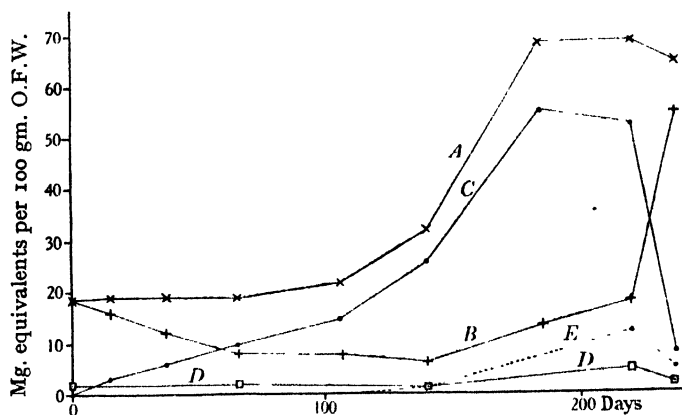


Fig. 1. Seasonal march in total malate content of whole plant, curve A (— x —); total malate in rhizome and roots, curve B (— + —); total malate in shoot, curve C (— · —); ammonium content of whole plant, curve D (— □ —); oxalate content of whole plant, curve E (— · —). All given as mg. equivalents per 100 gm. O.F.W.

The total "malate" analyses are summarised in Fig. 1. The three uppermost curves record the quantities in the whole plant, in the shoot (i.e. leaves), and in the rhizome+roots respectively expressed as mg. equivalents per 100 gm. O.F.W. The ages of the plants are given as abscissae. It will be noted that, on sprouting, the total quantity of acid in the leaves continues to increase and that in the rhizome and roots decreases. So that the total acid in the whole plant does not appreciably change in quantity. Much the simplest explanation of this result is, of course, that acid is translocated out of the rhizome into the expanding leaves.

In the height of summer (June, July and August) great increase

in the quantity of "malate" in the shoot, and some increase in the quantity in rhizome and roots, takes place. Finally, in autumn the leaves die down so that eventually there is of course no shoot and therefore no malate in the shoot; there is a very striking rise in acid content of the rhizome and roots at this time which clearly suggests that the greater part of the acid of the withering leaves is translocated to the rhizomes and roots.

(iv) *Seasonal march in total "malate" concentrations,
and in the fresh weights*

The analytical data of this section are the same as those of the last, but expressed on a different basis. Here they are given as mg. equivalents per 100 gm. fresh weight of the actual tissue examined: the results are plotted in Fig. 2. The upper curve gives the concentration in the shoot (i.e. laminae + petioles¹), and the lower curve gives similarly the average concentration in the rhizome and roots.

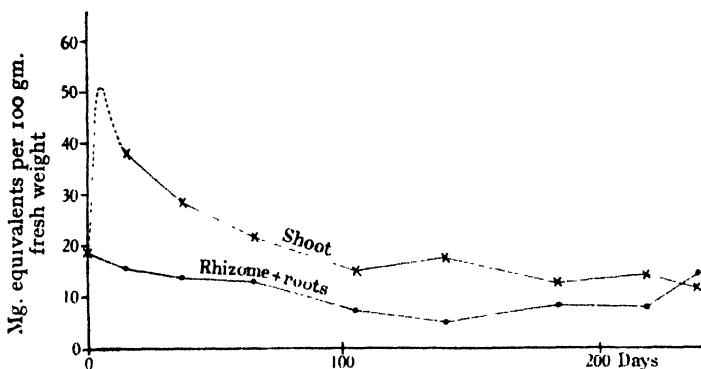


Fig. 2. Seasonal march in concentrations of total malate in shoot (— x —), and rhizome + roots (— · —). Given in mg. equivalents per 100 gm. fresh weight.

The first part of the upper curve is represented by a broken line. The existence of such a steep initial rise in acid concentration is not actually shown by the particular experimental results recorded in this section. Other experiments, however, indicate that the maximum concentration occurs in young leaves a day or so after the opening of the bud. Thus it will be seen that the acid concentration of the shoot decreases as the age increases fairly rapidly at first, but later in the

¹ The concentration in the petioles was found to be about 10–20 per cent. higher than that in the laminae.

year (during the summer and autumn) the change in acid concentration is slight.

The concentration in the rhizome decreases during the first half of the growing season, and increases again during the second half.

These results should be compared with the changes in the fresh weights of the different parts of the plant, which are shown graphically in Fig. 3. The fresh weights are expressed as multiples of the original fresh weight of the piece of rhizome from which the plant grew. A steady rather slow increase in fresh weight of the shoot occurs during the early part of the season, followed by enormous increase in fresh weight during the months of June, July and August.

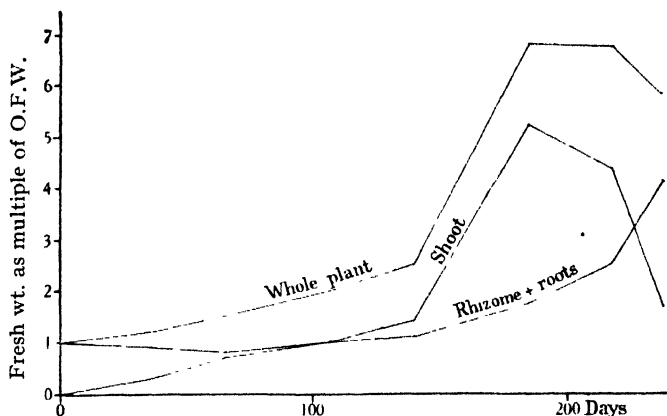


Fig. 3. Seasonal march in fresh weights of entire plant, shoot, and rhizome + roots. Given as multiples of the original fresh weight which is taken as unity.

The fresh weight of the rhizome + roots increases during the whole summer and autumn, and the most rapid increase occurs during the phase when the aerial parts are withering in late autumn.

Superficial consideration of the records given in Fig. 2 might lead one to conclude that active formation of acid occurred in the very young leaves and that no new formation of acid took place during the summer, and that translocation into the rhizome occurred in the autumn. These are the conclusions to which Ruhland and Wetzel (5, 6) have actually come. As pointed out above, the initial rise in concentration in the leaves is most simply explained as due to translocation of acid out of the rhizome. The fact that a comparatively small decrease in concentration of acid in the rhizome occurs as compared with the increase in the aerial parts is simply due to the very large

size of the rhizome compared with the young leaves (the relative fresh weights are given in Fig. 3 and also in Table IV).

The conclusion that no new formation of malic acid occurs during the summer during the period of most active carbohydrate metabolism is completely incorrect, as the most elementary observations show that enormous increase in fresh weight takes place, and this is accompanied by little or no decrease in "malate" concentration expressed on a fresh-weight basis.

In our view the maintenance of this nearly constant "malate" concentration is brought about by the occurrence of the equilibrium carbohydrate \rightleftharpoons "malic acid". In any case, it is evident that it involves the production of very large quantities of "malic acid" during the summer from other substances of which the source, it would appear, is the material formed during photosynthesis.

(v) *Seasonal march in oxalate and ammonium content*

Our analytical results are recorded in Fig. 1. The curve relating to oxalate content refers to the oxalate content of the aerial parts of the plant; the quantity present in the rhizome + roots was in all cases found to be exceedingly small or zero. In this our results agree with the statements of Ruhland and Wetzel. We do not, however, as they claim, find any simple relation between the quantity of oxalate formed in the older leaves and the quantity of either *l*- or *d*-malic acid which disappears from these leaves.

The curve relating to the quantities of ammonia refers to the quantity in the whole plant. It will be noted that comparatively little fluctuation in total quantity of ammonia takes place, and this quantity is very small compared with the quantity of malate. In this respect our results differ from those of Ruhland and Wetzel, who state that the molar concentrations of ammonium and *l*-malate are equal.

(vi) *Seasonal march in l- and d-malate contents*

The quantity of optically active malate was determined by making use of the abnormally high rotations of the molybdenum and uranium complexes formed by malic acid. The technique of Auerbach and Kruger (1) was followed in detail. The determination of the quantity of *d*-malate (and incidentally also of *l*-malate) depends on both total malate estimation and on the polarimetric determination:

$$\begin{aligned}\text{quantity of } d\text{-malate} &= \frac{1}{2} (\text{total malate} - \text{excess } l\text{-malate}) \\ \text{quantity of } l\text{-malate} &= \frac{1}{2} (\text{total malate} + \text{excess } l\text{-malate}).\end{aligned}$$

Our results are recorded in Fig. 4. The curves illustrate the seasonal march in excess *l*-malate of the shoot, and of inactive malate of shoot and rhizome + roots. All the acid in the rhizome + roots is inactive. The total "malate" analyses carried out by us do not distinguish between malic and citric acid. Ruhland and Wetzel stated that the inactive acid of the rhizome and shoot was *i*-malic acid; recent work carried out here, however, shows that considerable quantities of citric acid are also present. We do not know what fluctuations in citric acid content take place throughout the growing season, and are therefore not able to state the quantities of either *d*-malate or of total *l*-malate (if *d*-malate is present, the quantity of total *l*-malate will exceed that of excess *l*-malate).

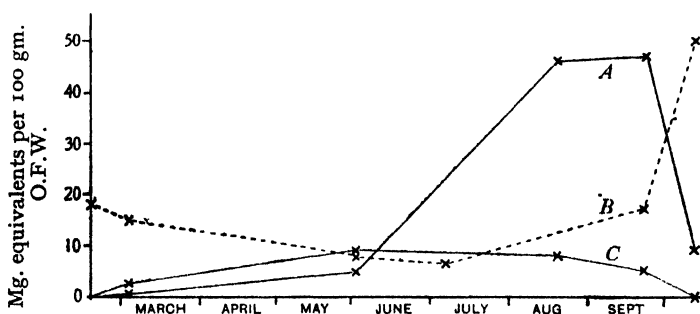


Fig. 4. Seasonal march of excess *l*-malate in shoot (curve A), inactive acid of shoot exclusive of oxalic acid (curve C), and inactive acid of rhizome + root (curve B).

It will be noted that the new malate formed in summer is apparently *l*-malate. Our results agree with those of Ruhland in showing that whereas *l*-malate leaves the withering shoot in autumn, optically inactive acid accumulates in the rhizome. Part of this inactive acid is citric acid, and the rest presumably is *dl*-malic acid as stated by Ruhland and Wetzel. We ourselves have not definitely identified it as such beyond doubt, and Ruhland and Wetzel do not state the method by which they identified it.

(vii) Seasonal march in titratable acidity

It is not proposed to record these experimental results in full, since they do not influence any conclusions drawn from other sections of the work. It may, however, be pointed out that throughout the seasonal cycle the titratable acidity varies from about one-third of the total acidity to about one-half of the total acidity. The low values

are found while the plant is in the resting phase from autumn to spring, but during the height of summer the titratable acidity accounts for a larger fraction of the total acid. The meaning of these results is of course that as new acid is formed during summer, bases are absorbed from the soil which partially neutralise the newly formed acid and cause the ratio of titratable to non-titratable acid to remain roughly constant.

DISCUSSION

The results recorded above show that the sprouting of resting rhubarb rhizomes is not accompanied by any increase in the quantity of either the "malate" or ammonium in the plant. A small part of the "malate" and ammonia pass out of the rhizome into the developing leaves, and owing to the small size of the latter the acid concentration becomes very high and a slight increase in the ammonium content is also noted. These facts are in complete opposition to the hypothesis that the mode of nitrogen transport from rhizome to developing leaves in rhubarb is of a peculiar type which justifies the classing of the rhubarb as an "ammonium plant" in contradistinction to the "amide plants". Ruhland and Wetzel claim that in many plants with highly acid sap (among them rhubarb) the amino acids leaving the storage organs are converted into equivalent quantities of *l*-malic acid and ammonia, and the ammonium *l*-malate so formed is transported to the region of protein synthesis. The actual experimental results on which this conclusion has been based are not given by Ruhland and Wetzel. So far as we can see, this conclusion must have been arrived at by erroneously regarding the high concentration of acid in the young leaves and petioles as evidence of an increase in total quantity of acid in the plant.

Similar erroneous reasoning could also be the source of Ruhland and Wetzel's statement that no further malic acid formation takes place during the summer, which is presumably based on the fact that no marked change in concentration occurs.

Ruhland and Wetzel (6) consider that the excess *l*-malic acid in the leaves arises *de novo* from amino acids, and that the *dl*-malate of the leaves is derived from a different source namely from the rhizome out of which it is translocated. Further the *l*-malate is eventually transported back to the rhizome where it is converted into *i*-malate (apparently the presence of citrate was not recognised).

We also observe this apparent "inactivation" of malic acid, and work at present being carried out here seems to indicate that it is a

relatively simple enzymatic process. Enzyme preparations have been obtained which "inactivate" *l*-malic acid but the mechanism is still quite uncertain.

Our results indicate clearly that malic acid is newly formed in rhubarb only during the summer months when photosynthesis is proceeding actively, and further that this acid is *l*-malic acid. In autumn this acid is translocated to the rhizome and converted to an optically inactive acid or acids among which is citric acid and possibly also *dl*-malic acid. This inactive mixture of acids is transported into the young leaves when the buds sprout in spring.

The evidence obtained by us so far does not enable us to conclude, as Steinmann(8) formerly concluded, that the acid was an intermediate product in the conversion of CO_2 to carbohydrate. It is, however, striking that *l*-malate only accumulates during the period of most active photosynthesis at the height of summer. It appears probable at present that the malic acid metabolism is intimately linked with carbohydrate, either in the processes of respiration or photosynthesis.

ADDENDUM

Some further data relating to the series of analyses dealt with are included in Table IV. These additional data do not affect the argument presented and are given merely to complete the description of the experimental work.

SUMMARY

1. Sprouting of rhubarb rhizomes is not associated with increase in quantity of ammonium or of "malate". There is therefore no reason to suppose that active deamination of amino acids with formation of ammonium malate occurs in connection with transport of nitrogen to growing parts. Translocation of "malate" from the rhizome to the young leaves does take place so that the concentration in the leaves rises.

2. The period of active *l*-malic acid formation occurs at the height of summer and not during the sprouting of the rhizome.

3. These facts support the view that malic acid is associated with carbohydrate rather than the protein metabolism.

4. The molar ratios of ammonia/malic acid are small at all times (usually about 0.1), and give no support to the hypothesis that malic acid is derived from the carbon residues of deaminated amino acids. This hypothesis demands that the ratio should be unity or more than unity.

TABLE IV
Schedule of main series of analytical results

Fresh weight, gm.				Total "malate", mg.-eq		
Date	Shoot	Rhizome + roots	O.F.W. rhizome	Shoot	Petiole	Rhizome + roots
Feb. 19	0	550	550	0	0	102
March 6	67	514	550	16.8	9.0	87.0
28	200	875	950	55.0	—	114.0
April 25	345	460	536	53.0	—	40.7
June 5	413	433	429	62.6	28.5	32.2
July 9	878	701	612	158.0	93.6	36.8
Aug 22	2260	759	437	240.3	166.0	56.8
Sept. 25	2719	1666	631	331.2	113.0	111.0
Oct. 15	692	1960	481	37.8	16.4	208.5
15	617	1782	434	35.2	—	308.1

Total l-malate, mg.-eq				
Date	Shoot	Rhizome + roots	Oxalate, whole plant	Ammonium, whole plant
Feb. 19	0	52	0	2.2
March 6	9.4	43.0	0	2.7
28	—	—	0	6.5
April 25	—	—	0	—
June 5	43.0	16.0	0	—
July 9	—	—	6.1	7.1
Aug 22	223.0	29.0	30.5	—
Sept. 25	315.0	55.5	76.9	32.8
Oct. 15	37.0	110.0	23.5	12.0
15	—	—	18.4	6.2

5. It is again emphasised that the term "malate" (in inverted commas) refers to acids of the malic acid group and includes citric acid. The inactive acid of the rhizome and leaf is certainly in part citric acid, though at certain seasons much of it may actually be, as Ruhland and Wetzel claimed, racemic malic acid.

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THE NEW EVIDENCE OF ISOSPORY IN PALAEOZOIC SEED PLANTS

By M. BENSON

(With 4 figures in the text)

AN intensive study of some sections of the Palaeozoic fructification *Schuetzia Bennieana* Kidston, kindly lent me by Dr Crookall after their return to the Geological Survey Museum, London, by Dr Halle, yielded results of twofold interest. A Note relating to them was published last July(3).

In the first place they considerably strengthened the evidence of the ovular nature of *Schuetzia*, as is shown in that Note and in a more recent paper where a revised diagnosis of *Calathiops* Goeppert is given(4).

In the second place, these female spores and embryo-sacs proved to be of a type unknown in the ovules of recent plants, and showed that the earliest ovules of which we have record of structure, i.e. those of the Lower Carboniferous horizon, bore female spores of the same size as their pollen grains. Not only were these spores of the same size and form but the female spores were as fully cuticularised as the male. Thus there had been no loss of the equipment for aerial transport on the retention of the spore in the seed.

As a result the young embryo-sacs had been regarded as pollen grains because the triradiate scar was still visible. It was not until the young tetrads of spores were found that these *Schuetzia* embryo-sacs (Fig. 2 a) were demonstrated not to be mere spores.

Since this first discovery was made several other species of Pteridosperm ovular apparatus of both Lower and Upper Carboniferous strata have been tested in the same manner, and the microtome sections of all those available at the Geological Survey Museum, London, have yielded in varying degrees of preservation minute spores or tetrads. The accompanying figures exhibit transverse and longitudinal sections of ovules in which separation of the carbonaceous matter of the non-cuticularised parts of the ovule has taken place. This carbonaceous matter is exhibited as black masses, almost entirely amorphous, on either side of the sectioned cuticularised

parts. The latter consist simply of the spores or embryo-sacs, and the epidermal layers of the cupules and ovules.

The cuticles are exceedingly tenuous, refringent layers determining the position of the wrappings of the spore—their epidermal sub-jacent cells explain the width which gives them a strap-like appearance in + or ↓ sections. Occasionally, when the section is oblique the wrapping layer looks wider. In some cases the epidermal cells are themselves cuticularised and, on separating partially, resemble bladders. The genuine spores demonstrate that Prof. Boyd Thomson's conclusions (7) as to the Isospority of Recent Seed Plants are confirmed by a study of Palaeozoic Seed Plants.

As space is limited I propose to confine this account to the description of such preparations as best illustrate the subject and have been selected for that purpose.

Fig. 1 shows parts of four sections on Halle's slide 3464. They are transverse of the younger group of ovules occurring on the same rachis as that examined by Kidston and reproduced from his *Memoirs* (6) in our Fig. 2 c. Such ovular, bud-like bodies are now included in the form genus *Calathiops* Goeppert, so that we may call this body *C. Schuetzia Bennicana*. Fig. 1 a, b, c are successive sections (Row I, 2, 3, 4) on this slide 3464. In two drawings the carbonaceous matter has been omitted.

Fig. 1 a shows what I regard as a degenerating spore. Fig. 1 b shows the spore which would have given rise to the embryo-sac with possibly a residuum above it. Fig. 1 c shows the reduced size of the ovule presumably above or below the sporogenous region. Fig. 1 d is a complete tetrad of female spores. This is assumed, as there is no trace of a spore in the sections before or after Row I, 7 in which this tetrad occurs. All the drawings were made with a camera lucida and the magnification is shown by the line representing 19–20 μ.

Fig. 2 a and c are reproduced from Kidston's *Memoirs*, Pl. CVII, figs. 9–13 (6) and represent the *Calathiops* (*Schuetzia*) *Bennicana* fructification "c" from which he procured the six bodies "a" by maceration. They are magnified a little less than the pollen grain beside them. Two of the larger bodies show a triradiate scar no larger than that of the female spore in Fig. 1 b. The true ♀ spore is precisely similar to the pollen grain in external appearance which is given at b. It is interesting to note that the body c was procured by Kidston from the same specimen as that which provided Halle with the material for his slide 3464 which yielded the minute spores in Fig. 1.

Fig. 2 b is one of many which are available from the Burntisland

structural material of *Heterangium Grievii*. The one drawn by camera lucida here occurs in R.H.C. Coll., C.N. 270, 1, and is among those of Fig. 3 in the *Heterotheca* paper(2).

Fig. 3 *a*, *b* are transverse sections of an embedded piece of *Whittleseya elegans* Newb., and are figured from Halle's slide 558.

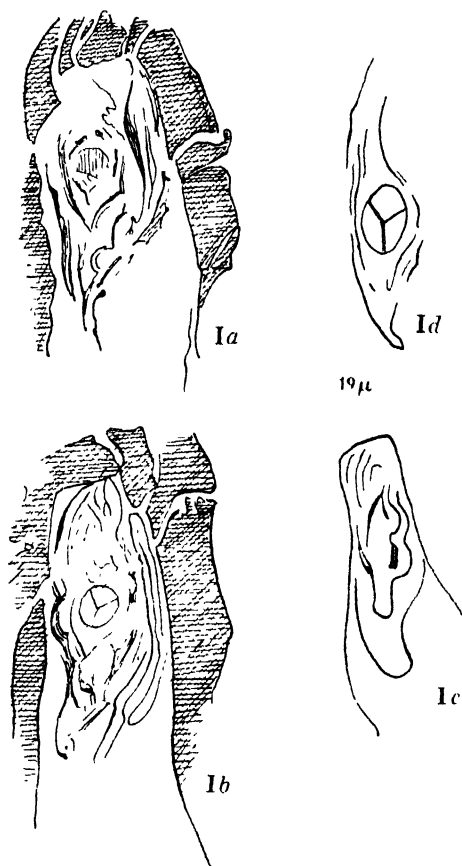


Fig. 1.

They exhibit two tetrads ensheathed in the epidermal layers of the cupules and seeds. Though in two successive sections (Row I, 2 and 3) they represent the spores of distinct seeds, as they occur in different parts of the sections. The tetrads have had the walls separating their constituent spores dissolved by the drastic treatment to which they were subjected when the fossil was being softened for embedding purposes (vide(5), pp. 5-7). The tetrad in Fig. 3 *b* has begun to

germinate but that of 3 *a* is similar in size and form to the tetrad shown in Fig. 1 *d*, except that it shows none of the inner walls.

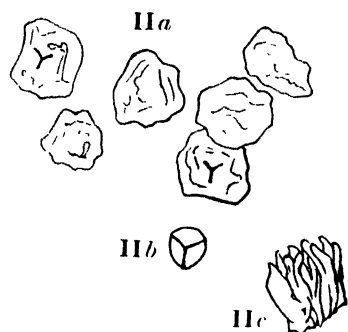


Fig. 2.

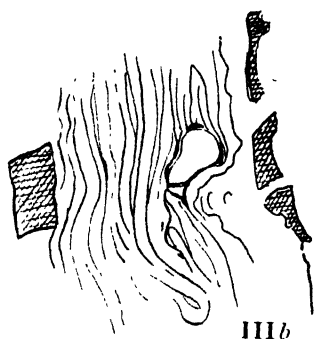
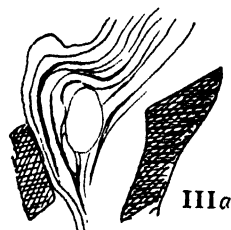


Fig. 3.

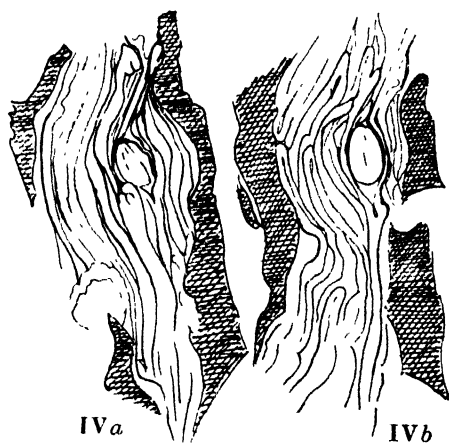


Fig. 4.

Fig. 4 *a, b* are also sections from an embedded piece of *Whittleseyia elegans* Newb., but they are cut in the longitudinal plane. In these

consecutive sections (Slide 360, Row II, 1, 2) the same tetrad is represented in two pieces, showing that germination had probably started as in Fig. 3 *b*. The average linear dimensions are computed easily from the 19–20 μ line which serves equally for all the sections and for the pollen grain in Fig. 2 *b*.

Both Figs. 3 and 4 are examples of Upper Carboniferous structures, as *Whittleseya elegans* occurs in Westphalian rocks.

N.B. All the microtome sections containing the female spores described in this paper were made by Dr T. G. Halle, who employed his new method described on pp. 5–7 of his treatise(5). It was at his suggestion that I procured the loan of these valuable slides.

SUMMARY

Examples are described from both Lower and Upper Carboniferous rocks of specimens of *Calathiops* (Goeppert) fructifications. They are shown to be immature ovule apparatus which exhibits minute, triradiate ♀ spores of 19–20 μ in linear dimensions.

This is shown to be the linear dimension of the pollen grain of *Heterangium Grievii* which occurs not only in the pollen sac *Heterotheca Grievii*(2) but in the pollen chamber of *Sphaerostoma ovale* which is accepted as the ovule of *Heterangium Grievii*(1).

Pollen grains similar to the above are found in Westphalian rocks, *vide* Pl. CLIII, fig. 4 of Kidston's *Memoirs*(6).

Four figures are given: Fig. 1 *a, b, c, d*; Fig. 2 *a, b, c*; Fig. 3 *a, b*; Fig. 4 *a, b*. These are fully described in the text.

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STUDIES IN THE EFFECTS OF PROLONGED ROTATION OF PLANTS ON A HORIZONTAL KLINOSTAT

I. GROWTH RATE

By E. D. BRAIN, F.L.S.

(With 5 figures in the text)

DURING experimental work on the geotropism of certain seedlings (Brain (2)), it was found that *Lupinus polyphyllus* seedlings grown entirely on the klinostat showed an alteration in the presentation time for the hypocotyl and a change in anatomical structure. It seemed possible therefore, that if continuous growth on a klinostat could cause such changes in a seedling, a further examination extending over a wider range might yield results which would help to elucidate the vexed problem of the mechanism of geotropic perception and response.

This paper is the first of a proposed series dealing with the effects of prolonged rotation of plants on a horizontal klinostat, and is a study of the growth rate of *Narcissus pseudo-narcissus*, *Asplenium bulbiferum* and various seedlings on the klinostat.

It is proposed to deal with anatomical and physiological investigations of these plants in later papers.

INTRODUCTION

The fact that the mechanism of tropic curvature involved differential growth on the opposite sides of the curving organ and that the rate of growth, as well as its direction, was affected by gravity, was demonstrated by Sachs (26, 27), who noted a marked increase in the growth of the underside of horizontally placed shoots and the upper side of horizontally placed roots.

Vöchting (29) noted the decrease of shoots on inversion and differences in the growth rate of upward and downward directed branches of weeping trees. Elfving (7) could find no change in the growth rate of seedlings or *Phycomyces* on the klinostat, although an increase in growth of grass haulms had been noted (cf. Pfeffer (21),

p. 110). Luxburg in 1905 re-examined the growth of plants on the klinostat. He found no alteration in the growth of nodes of *Tradescantia fluminensis*, *T. virginica* and *Gallium rubiodes* on a klinostat (17). The theory of "Light Growth Reaction" put forward by Blaauw in 1914 (1) as an explanation of phototropism was examined in relation to geotropism by Zollikofer (32). She found that the course of geo-growth reaction shows a close similarity with that of light growth reaction in *Avena coleoptiles*. Her results were contradicted by Konigsberger (13), who found no alteration in growth on the klinostat and concluded that plants do not perceive gravity when rotated on a klinostat nor show a geo-growth reaction. Several other workers have examined the growth of *A. coleoptiles* on the klinostat. Bremekamp (3) does not regard the decrease in length recorded for *A. coleoptiles* on the klinostat as an alteration in growth but rather as the result of a curvature which the coleoptile makes after several hours' rotation on the klinostat. Dolk (6) could find no alteration in the growth of *A. coleoptiles* after being rotated on a klinostat. These results will be referred to in more detail later (p. 105).

The object of these experiments has been to compare the growth of plants when they are slowly rotated on a horizontal klinostat over considerable periods with that of plants in the normal position, under the same conditions of temperature, humidity, etc. In this way the growth of fronds of *Asplenium bulbiferum*, leaves and peduncles of *Narcissus pseudo-narcissus*, and different species of seedlings has been examined. *Asplenium bulbiferum* was chosen because a previous study has been made of the normal growth and geotropic behaviour of this species (Pranker (23, 24), Waight (30)).

METHODS

The ferns and seedlings were grown in a greenhouse at a temperature of about 20° C. and 75–90 per cent. humidity. *Narcissus* bulbs were grown in a room which could not be kept at such an even temperature. Careful records of maximum and minimum temperatures were kept in all cases. The ferns were grown in pots and the bulbs and seedlings in cardboard cartons in damp moss. Equal quantities of water were given to each set of plants during experiment. The klinostats rotated once an hour. All measurements were taken with a centimetre rule.

RESULTS

(I) *Narcissus pseudo-narcissus*

Qualitative effects of the klinostat are shown in the developing shoot, and later in the inflorescence. The normal shoot is slightly curved when it first breaks through the bulb. This is rectified as growth proceeds in the upright position, but in bulbs on the klinostat the shoots become curved and do not straighten out for two weeks or more, the growth proceeding in the direction in which it starts. It is well known that the flower-bud does not make the usual bend in the receptacle on the klinostat but remains in a straight line with the peduncle. The flowers of bulbs on the klinostat developed and withered more rapidly than upright ones.

The daily growth of leaves and peduncles of six bulbs on a klinostat and of six bulbs upright was measured. Both leaves and peduncles show an increasing growth rate with development. In the upright leaves a maximum of 1.0 cm. per day is reached by the third week in leaves over 15 cm. in length. On the klinostat growth increases more rapidly, in the second week being 0.96 cm. and in the third week 1.3 cm. per day, after which it decreases to 0.55 cm. per day. A similar effect is shown in peduncles on the klinostat, in which growth rate is greater for the first two weeks and the flowers open and wither more rapidly than the upright ones (see Tables I and II).

When compiling the tables for *Narcissus pseudo-narcissus* and *Asplenium bulbiferum* the average growth rate per day for each leaf or peduncle was calculated and the mode taken for each series of leaves, e.g. third week on klinostat; twenty-one leaves used. The average growth rate per day was calculated for each leaf and the resulting averages classified in a frequency table and the mode calculated according to the formula:

$$Mo = l + \frac{f_2}{f_2 + f_1} \times i,$$

when l = lower limit of modal class, f_1 = frequency of class next below class, f_2 = frequency of class next above class, i = class interval (see Mills (17)).

Results classified

Average growth rate in cm. per day	Frequency
0.7-0.99	0
1.0-1.29	16
1.3-1.59	5
1.6-1.89	0

$$Mo = 1.0 + \frac{5}{5+0} \times 0.3 = 1.3.$$

TABLE I

Narcissus pseudo-narcissus leaves

Date	Temperature ° C.		Height in cm.	Average growth rate in cm. per day	
	Max.	Min.		Upright	Klinostat
Feb. 3-20	64	44	0-7.5	0.4	0.4
			7.6-15	0.75	0.96
Feb. 21-27	67	53	15+	1.0	1.3
Feb. 28-March 6	66	48	15+	1.0	0.55
March 7-13	62	49	15+	0.4	0.35
March 14-19	66	50	15+	0.1	0.0

Twelve bulbs and thirty-six leaves used.

TABLE II

Narcissus pseudo-narcissus peduncles

Date	Temperature ° C		Average growth rate in cm. per day	
	Max.	Min.	Upright	Klinostat
Feb. 9-15	59	44	0.3	0.65
Feb. 16-21	64	44	0.9	1.35
Feb. 22-28	67	53	1.9	1.75
March 1-7	64	48	1.1	0.48
March 8-15	61	50	0.1	0.0

Eleven peduncles used.

These results are presented graphically in Fig. 1.

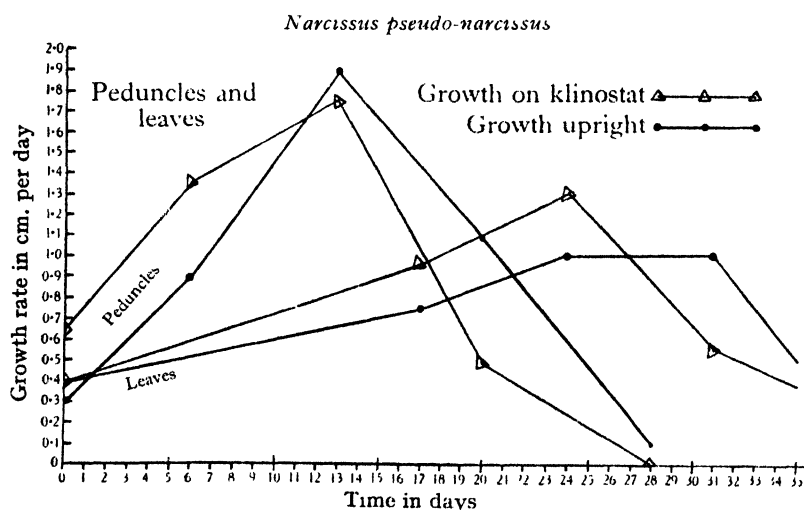


FIG. 1. Graph to show growth rate of *Narcissus pseudo-narcissus* peduncles and leaves on the klinostat and upright.

(2) *Asplenium bulbiferum fronds*

Growth rate was measured of fronds developing entirely on the klinostat and of control plants under the same conditions in a greenhouse of which the temperature was kept at 20° C. and the humidity 75-90 per cent.

Results show a grand period of growth which is coincident with the developing stages of the frond, maximum growth rate being shown during the adolescent stage (Waight (30)). The terms used to denote the different stages are similar to those used previously (Prankerd (23, 24), Waight (30)).

I. Infant stage with leaflets in tight apical coil.

L.I. Late Infant stage with leaflets in loosening apical coil.

A. Adolescent stage with leaflets unfolding below coil.

M. Mature stage with all leaflets unfolded.

The epinastic movements of the fronds were observed, and except for the fact that the frond may take up a position at a greater angle from the main axis of the plant on the klinostat, at the beginning of its growth, the subsequent epinastic curvatures are similar on and off the klinostat.

TABLE III

Asplenium bulbiferum fronds

Average growth per day in cm.

Stage of frond	Average growth per day in cm.		Difference on klinostat
	Upright Series I.	Klinostat February-March	
I.	0.4	0.5	+0.1
L.I.	0.4	0.6	+0.2
A.	0.7	1.25	+0.55
M.	0.3	0.4	+0.1
Series II. May			
L.I.	0.9	1.0	+0.1
A.	0.95	1.5	+0.55
M.	0.55	0.4	-0.15

Twenty-four plants and thirty-nine fronds used.

These results are presented graphically in Fig. 2. The length of time which each stage lasts is shorter for fronds on the klinostat than in the normal position.

Table IV shows the average length for each stage in days.

The difference in time being more marked in the infant stage of the frond indicates the effect of the rotation on the klinostat on the epinasty of the frond which involves the unrolling of the apical coil

TABLE IV

Asplenium bulbiferum fronds

	Stage ...	I.	L I.	A.	M.
Average time in days:	Upright	12	7	10	7
	Klinostat	7	6	9	7
	Difference	5	1	1	0

rather than the lengthening of the rachis at the infant stage. The figures in Table III show that a definite increase in the growth occurs in plants on the klinostat. It will be seen that the daily growth rate is

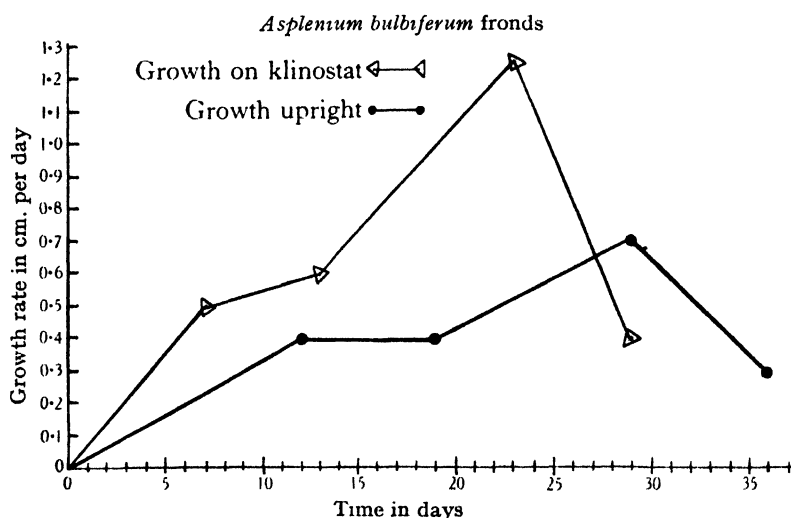


Fig 2. Graph to show growth rate of *Asplenium bulbiferum* fronds on the klinostat and upright.

slightly lower for the first series in February and March than for the second series in May; but, it is interesting to note that the difference between the two sets is identical for the A. stage in both series (0.55 cm. per day). Another most significant point to be noted is that the greatest difference in the growth rate occurs at the stage at which the frond is most sensitive to gravity and the presentation time is at its lowest value (Waight (30), p. 58). In Table V the values for the presentation times are taken from Waight's paper.

Fig. 3 shows these relationships in graphical form, the relative sensitivity to gravity being plotted as the reciprocal of the presentation time for each stage.

TABLE V
Asplenium bulbiferum fronds

Stage of frond	Presentation time in hours	Difference in growth in cm. per day
I.	3-8	0.1
L.I.	2	0.2
A.	0.5-1.5	0.55
M.	2.5-6	0.1

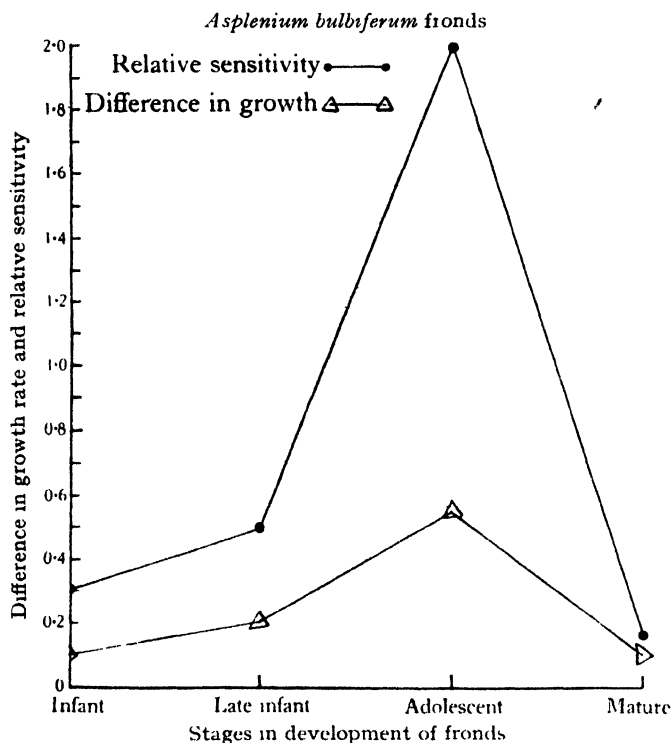


Fig 3 Graph to show relation between the stage of development of the frond, relative sensitivity to gravity and the difference in growth rate on the klinostat and upright, in *Asplenium bulbiferum*. Relative sensitivity is plotted as the reciprocal of the presentation time for each stage.

(3) Seedlings

Seeds of *Lupinus albus*, *Helianthus annuus*, *Avena sativa* and *Zea mays* have been grown from germination on the klinostat. The seeds were all soaked for 24 hours in water and planted in damp moss in cardboard cartons, some of which were placed on klinostats and others

kept upright as controls. It was thought advisable to test the germination on a vertical klinostat, revolving at the same rate as the horizontal ones (one revolution per hour). This was carried out with *Lupinus albus* seedlings, and no difference between growth of those on the vertical klinostat and the control plants was found either in radicle or hypocotyl. Measurements of the growth of several hundred seedlings in repeated series have shown consistent results for each species. The results recorded in Tables VI–XI are typical of many other series of experiments.

TABLE VI

Lupinus albus. Average length in cm.

Time in days	Upright		Klinostat		Difference on klinostat	
	Root	Shoot	Root	Shoot	Root	Shoot
3	3.1	1.2	2.7	1.1	–0.4	–0.1
4	4.1	1.5	3.7	1.4	–0.4	–0.1
6	7.0	2.1	6.5	1.8	–0.5	–0.3
8	9.6	3.2	8.8	3.1	–0.8	–0.1
9	—	3.78	—	3.95	—	+0.17
11	—	5.3	—	4.9	—	–0.4
14	13.6	7.4	9.6	6.7	–4.0	–0.7

Lupinus albus radicles show decreased growth on the klinostat, but it will be seen from Table VI that *L. albus* does not show increased growth of the hypocotyl when the seeds are grown on the klinostat. However, if seeds are grown normally until hypocotyls are 3.0–4.0 cm. in height and then placed on a klinostat, an increase in growth rate is shown. Whether the lower growth of the hypocotyl in the klinostat seedlings is due to the decreased root volume or to adaptation to their condition is not clear. The following figures show the alteration in the growth rate produced by placing seedlings on the klinostat after they have started growth normally.

TABLE VII

Lupinus albus. Average growth rate in cm. per day

Time in days	Root		Shoot	
	Control	Klinostat	Control	Klinostat
Previous days all upright	1.4	1.4	0.8	0.7
1	1.4	0.7	0.8	1.1
2	0.9	0.8	1.2	1.4
3	1.9	1.0	0.5	0.9
4	2.1	0.8	0.86	1.0

If the seedlings are placed on the klinostat on alternate days an increased growth in hypocotyls on the days on the klinostat is marked.

Similar experiments with radicles show a decrease in growth on the klinostat (see Table XII and Fig. 5, p. 108).

The results recorded in Table VIII show a decrease in the growth of *Avena* roots on the klinostat and no difference in growth of coleoptiles. The first leaf grows less rapidly on the klinostat.

TABLE VIII

Avena sativa. Average length in cm.

Time in days	Upright		Klinostat		Difference on klinostat	
	Root	Shoot	Root	Shoot	Root	Shoot
3	2.8	0.5	2.2	0.45	-0.6	-0.05
4	—	1.35	—	1.4	—	+0.05
6	7.7	3.1	7.0	3.1	-0.7	0.0
8	—	3.4	—	3.4	—	0.0

The growth of *Avena sativa* coleoptiles on the klinostat has been the subject of much research (Zollikofer, Königsberger, Bremekamp, Lange, Dolk etc.). Zollikofer⁽³²⁾ showed fluctuations in the growth rate after short periods of stimulation on the klinostat, which she regarded as a demonstration of a geo-growth reaction comparable to the light-growth reaction of Blaauw. Königsberger⁽¹³⁾ and Dolk⁽⁶⁾ found no alteration in growth of coleoptiles on the klinostat, and Bremekamp⁽³⁾, using special photographic methods for measuring growth, showed a decrease on the klinostat. This he explains as due to the curvature made by coleoptiles after several hours' rotation. Lange^(15, 16) has now shown this curvature to be photonastic and regards it as the behaviour of a dorsiventral organ on the klinostat, similar to that described by Kniep for leaves of *Lophospermum*⁽¹¹⁾. Went⁽³¹⁾, in his research on growth regulators in *Avena* coleoptiles, found no alteration in their production in the coleoptile tip after rotation on the klinostat for 25, 40 and 50 min. From which fact he concludes that a geo-growth response would not occur under similar circumstances. Cholodny⁽⁵⁾ found no alteration in growth of coleoptiles of *Avena* on changing their position from vertical to horizontal.

TABLE IX

Helianthus annuus. Average length in cm.

Time in days	Upright		Klinostat		Difference on klinostat	
	Root	Shoot	Root	Shoot	Root	Shoot
4	2.6	0.95	2.4	0.9	-0.2	-0.05
6	6.2	1.76	6.3	2.45	+0.1	+0.69
8	8.3	3.5	8.8	5.4	+0.5	+1.9
11	—	5.2	9.2	6.4	—	+1.2

Helianthus annuus seeds germinated more quickly on the klinostat and the hypocotyl was straight, without the usual hook. Seedlings germinated normally and then placed on the klinostat remained hooked about six days after germination and straightened out again before the control seedlings. The hypocotyls grew more rapidly on the klinostat than upright after a height of 0.5–1.0 cm., below which there seems no difference. Hawker (8) has shown that the straightening out of the hook of the hypocotyl is associated with the development of statoliths at the tip. It seems as if the hook is partly due to positive geotropism and also involves hyponasty, since the straight hypocotyls bend after germination on the klinostat. The radicles of *H. annuus* show a decrease in growth on the klinostat until they are 5.0 cm. in length when side roots develop. These grow more rapidly in the upright seedlings than in the klinostat plants, in which the main root continues to grow on, and the side roots are smaller. Thus on the klinostat plants are produced which have longer main roots and fewer smaller side roots than the upright plants. The effect of the klinostat seems to decrease root growth by retarding development of the side roots, although the continued growth of the main root in klinostat plants makes it appear as if there was greater growth of roots (see Table X and Fig. 4).

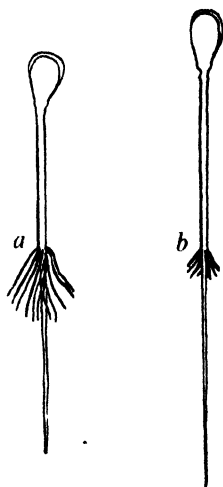


Fig 4. *Helianthus annuus* seedlings. a, grown upright; b, grown on the klinostat.

TABLE X

Helianthus annuus. Average length in cm.

Time in days	Upright		Klinostat		Difference on klinostat	
	Root	Shoot	Root	Shoot	Root	Shoot
2	0.38	—	0.32	—	-0.06	—
3	3.0	0.51	1.4	0.64	-1.6	+0.13
4 (side roots develop)	4.0	1.0	3.4	1.0	-0.6	0.0
5	5.5	1.4	5.6	1.6	+0.1	+0.2

Cholodny (4) has found no alteration in growth in *Helianthus* and *Lupinus* hypocotyls on changing their position from vertical to horizontal.

TABLE XI

Zea mais. Average length in cm.

Time in days	Upright		Klinostat		Difference on klinostat	
	Root	Shoot	Root	Shoot	Root	Shoot
4	0.96	0.5	1.5	0.9	+0.54	+0.4
6	3.8	1.4	4.1	1.8	+0.3	+0.4
8	6.4	3.0	6.8	3.1	+0.4	+0.1
11	7.0	—	10.3	—	+3.3	—

Zea mais shows increased growth of the coleoptile and leaf for the klinostat and differs from the other species examined in showing increased growth of the root on the klinostat. It is interesting to note that other workers have found that *Zea mais* radicles behave differently on geotropic stimulation. Keeble, Nelson and Snow (10) record increased growth in *Zea mais* radicles after six hours horizontality. This was not confirmed by Navecz (20), who also could not find any alteration in the growth of *Lupinus albus* roots or *Avena* coleoptiles. Porodko (22) studied the growth of *Zea mais* radicles and found that they usually grew at an angle to the vertical, which varied according to the position of the seed, and that on the klinostat they behaved as dorsiventral organs, taking up an angle of 20° from the main axis. Their behaviour on the klinostat is therefore explicable on these grounds, the radicle not being ortho-geotropic as that of *Lupinus albus* or *Helianthus annuus* but plagiotropic and subject to different geotropic reaction in the horizontal position.

(4) Experiments with alternate periods on the klinostat and upright

Table XII shows the results of a series of experiments in which the plants were placed on the klinostat on alternate days so that the growth could be compared in the two positions and with a series of control plants growing normally. Fig. 5 shows these results graphically.

DISCUSSION OF RESULTS

It seems probable from the experiments recorded above that rotation on a horizontal klinostat has a differential effect on the growth of root and shoot, the former showing an increased and the latter decreased growth. This opposite effect is nicely demonstrated in the experiments in which plants were rotated and kept upright on alternate days (Fig. 5). *Asplenium bulbiferum* fronds show the greatest difference in growth at the stage of greatest sensitivity to gravity. This is for the adolescent stage of the developing frond when

TABLE XII

Alternate days on klinostat

Time in days	Temperature		Growth rate in cm. per day			
	Max.	Min.	Upright	Klinostat	Control	
	<i>Narcissus pseudo-narcissus</i> leaves					
1	60	53	—	1.3	0.9	
2	56	50	1.0	—	0.9	
3	60	54	—	1.0	0.9	
4	56	50	0.7	—	0.9	
5	57	48	—	1.12	0.9	
6	63	51	0.4	—	0.6	
7	62	52	—	0.7	0.3	
<i>Asplenium bulbiferum</i> fronds						
Stage L.I.	1	74	68	0.9	—	0.8
	2	74	68	—	1.0	
	3	72	68	0.9	—	
	4	74	68	—	1.4	
	5	73	68	0.9	—	
	6	68	60	—	1.4	
	7	70	60	0.7	—	
Stage A.	1	74	68	—	1.6	1.1
	2	74	68	1.0	—	
	3	72	68	—	1.3	
	4	74	68	1.0	—	
	5	73	68	—	1.4	
	6	68	60	0.9	—	
	7	70	60	—	1.4	
<i>Lupinus albus</i> hypocotyls						
1	72	56	—	1.2	0.95	
2	70	60	0.9	—	0.85	
3	72	60	—	1.6	1.4	
4	70	60	1.2	—	1.4	
5	67	63	—	2.0	1.0	
6	70	63	0.9	—	0.9	
<i>Lupinus albus</i> radicles 8-hour periods						
1	74	69	1.37	—	0.8	
2	70	70	—	0.87		
3	70	69	1.1	—		
4	70	70	—	0.67		
5	70	68	0.8	—		
6	72	68	—	0.4		
7	74	72	1.0	—		

the leaflets are unfolding. The presentation time is at its lowest value, 0.5–1.5 hours, and there is a difference of 0.55 cm. between the daily growth rate on the klinostat and upright.

In *Narcissus pseudo-narcissus* peduncles the greatest difference occurs at the stage when the bend of the receptacle normally occurs. In *Avena* coleoptiles and leaves and *Zea mays* radicles growth on the klinostat has different effects from that of *Lupinus albus* and *Helianthus annuus* seedlings, which may be correlated with the plagiotropism of the former seedlings.

Do these facts bear any relation to the geo-growth reaction and growth regulator theories of geotropism? Various theories of geotropic reaction have been put forward in recent years. Renner (25) speaks of a characteristic radial polarity, dependent on the position of the

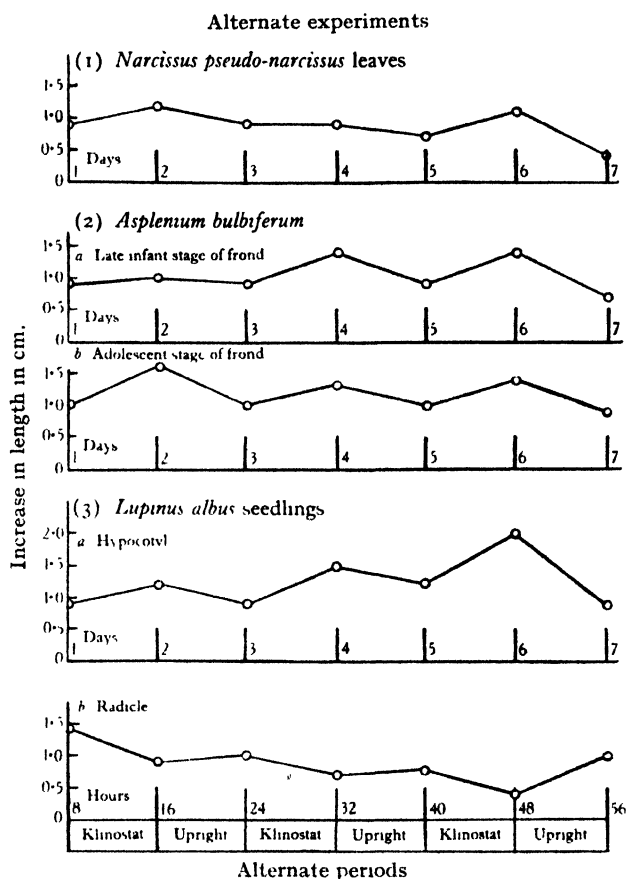


Fig. 5. Graphs to show growth rate during alternate periods on the klinostat and upright in *Narcissus pseudo-narcissus* leaves, *Asplenium bulbiferum* fronds and *Lupinus albus* hypocotyls and radicles.

tangential walls, which causes growth reaction and resultant curvature. He considers radial polarity of root and shoot is not only dependent on special structures such as statoliths but is also due to direct stimulation of plant organs through polarity. Metzner (18) traces geotropic reaction to an inherent curvature tendency related to

epinasty and hyponasty. Snow⁽²⁸⁾ endorses the theory of other workers (Cholodny⁽⁵⁾, Dolk⁽⁶⁾, Went⁽³¹⁾, Hawker⁽⁹⁾), and describes geotropic response as due to growth-regulating substances which form at the tip in shoots and accelerate cell extension and elongation below, and at the root tip, retarding growth above, thus forming the basis for the opposite tropisms of root and shoot. While suggesting that the opposite effects of the klinostat on the growth of root and shoot appear to endorse this, I should like to point out that the experimental basis for the theory seems unsatisfactory. Knowing from bitter experience the extreme sensitivity of root tips, the least touch causing traumatic curvature, I find it difficult to accept the evidence of experiments which are wholly dependent on cutting off the tips of roots and shoots and replacing them by means of gelatine, and it is difficult to feel convinced that after such surgical operations resultant curvatures are purely geotropic and can be ascribed to the growth-regulating substance which has diffused through the gelatine block. It is therefore satisfactory to find that further support has been given to the theory by experiments which do not involve decapitation and wounding. Laibach and Kornimann⁽¹⁴⁾ have found alteration in growth and curvatures in *Avena coleoptiles* after the application of agar blocks, containing growth hormone, to the epidermis of intact coleoptiles. Kögl, Haagen-Smit and Hanni Erxleben⁽¹²⁾ have grown seedlings in solutions of extracted hormone of different concentrations and found marked decrease in root growth according to the concentration of the solution into which the roots were placed. The latter results make an interesting comparison with the results recorded above which show a decrease in the growth of radicles on the klinostat.

Additional evidence for the growth regulator theory may be found in the demonstration of a growth reaction produced by a continuous all-sided geotropic stimulus, in plants otherwise growing under natural conditions. The fact that this effect varies according to the tropic tendency of the plant organ and has been demonstrated above in varied types of plants seems compatible with a geo-growth reaction comparable to the light-growth reaction of phototropism.

SUMMARY

1. *Asplenium bulbiferum* fronds and *Narcissus pseudo-narcissus* leaves and peduncles grown on the klinostat continuously for several weeks show an initial increase in the growth rate on the klinostat. The

difference in growth is most marked in the most sensitive stage of the *Asplenium bulbiferum* frond to gravity and at the stage when the *Narcissus* flower receptacle normally bends.

2. Seedlings of *Lupinus albus*, *Helianthus annuus*, *Avena sativa* and *Zea mais* germinated and grown on the klinostat showed decreased growth of the radicle in the first three species on the klinostat. *Helianthus annuus* hypocotyls are not hooked on the klinostat until about six days after germination, and they show an increase in growth on the klinostat. Up to 5.0 cm. growth of radicles is less than that of controls, and side roots are fewer and grow more slowly. On the klinostat the main root continues to grow, while in the controls its growth slows down after the production of side roots. *Avena sativa* shows no difference in the growth of the coleoptiles, but the first leaf grows more slowly on the klinostat. *Zea mais* differs from the other species in showing marked increase in growth of the radicle on the klinostat. This is possibly due to plagiotropism. The coleoptile and first leaf grow more quickly on the klinostat than upright. *Lupinus albus* hypocotyls do not show increased growth if they are germinated on the klinostat. If grown normally until 3.0 cm. high and then put on the klinostat they show increased growth rate.

3. *Narcissus* leaves, *Asplenium bulbiferum* fronds and *Lupinus albus* hypocotyls placed on the klinostat on alternate days show increased growth on the klinostat. *L. albus* radicles placed on the klinostat alternately for eight-hourly periods show marked decrease in growth on the klinostat.

4. These results appear to support the theory of geo-growth reaction and demonstrate the fact that a differing growth mechanism is involved in the opposite tropisms of root and shoot.

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ON THE CONCEPTS OF PLANT MORPHOLOGY WITH SPECIAL REFERENCE TO VIEWS AND COMMENTS ON THE NATURE OF THE ANGIOSPERMIC STIGMA

By H. HAMSHAW THOMAS

SACHS in his *History of Botany* wrote: "The physiological arrangements in vegetable organs are not obvious to the eye; they must be concluded from certain incidental circumstances or logically deduced from the results of experiments. But experiment presupposes the proposing a definite question resting on a hypothesis, and questions and hypotheses can only arise from previous knowledge." The general principle embodied in this statement is applicable equally to morphological research, and especially to the problems of the stigma, which are both physiological and morphological. The writer's recent paper (10) on this subject was intended, as was stated, to open up new lines of thought and investigation. It was put forward, as clearly stated, not as a conclusion but as a hypothesis founded on some previous knowledge, requiring, however, both experiment and the collection of further information to show whether it had any permanent value. It was intended to provoke discussion, but still more to lead botanists to the much neglected study of the stigma. Now almost all our present knowledge of this structure has come from morphologists, but after studying and teaching plant morphology for more than twenty-five years the author had reached the view that little real progress will be made in the morphological study of the flowering plants until the foundations of our ideas are examined and formulated. This led to an attempt to formulate a general scheme, though it is quite open to critics to show either that such a formulation is unnecessary or to provide a better one.

The criticism published in a subsequent number of this journal (11) is welcome because it displays another view-point though disappointing because it avoids the real difficulties, but it illustrates the need for consideration of the basis of morphological argument. It shows how difficult it is for those who are studying the same subject to understand each other's view-points, and to evaluate the opinions put forward in the absence of any recognised technique for demonstrating the validity of morphological deductions. My critic seems to

have been so much occupied in his search for inconsistencies that he has lost sight of the fact that no morphological view can last which is devoid of reasoned foundations. Since he also seems to have abandoned the classical view of the carpel he ought to establish the basis of his own concepts. He might have shown that he had a broader and surer foundation for his ideas, which in certain respects, viz. the view that the ovules rather than the carpels are the fundamental elements of the gynaeceum, resemble those of the present writer. Since he seems to neglect the essentials of morphological argument, a detailed reply to the criticism would have little permanent scientific value, and hence a further consideration of the principles involved seems the most useful form of answer.

Prof. McLean Thompson considers the writer's approach to the complex problem improper. He finds obscure the bearing of fundamental propositions on the question of carpel evolution, which seems to imply that he is prepared to outline the possible history of a great group of plants without any general ideas of the course of plant evolution or of the facts of heredity. The special study of the "problems of modern flowering" (which he does not specify) possibly renders such considerations unnecessary, especially if "each form of flower may be accepted as an individual expression of the problem of flowering".

While dismissing my preliminary considerations as irrelevant, our critic states that "he believes that by the study of living Angiosperms the essential problems of angiospermy may be exposed. The foundation of this belief is observation from the floras of many lands." By such observation Prof. Thompson has reached certain tentative views which he has summarised. Is it improper to suggest that these views are really based on many other considerations which are not stated? It was observation of many floras of many lands that led Robert Brown to the conclusion that each simple carpel has two lateral stigmata. The same observation led Engler, Wettstein, Lotsy, Hutchinson and others to suggest different views as to the possible evolution of the varied forms of flowers, views which differ from those of our critic. It may be here remarked that the writer has not seen any modern published system of taxonomy, even that of Hallier, which does not rest mainly on floral structure, and in which the author abandons the view that the parts of the flower represent modified leaves. It is not every author who, like Hutchinson (6), p. 8, states clearly that his system is the logical interpretation of the theory that the parts of the angiospermous flower are modified leaves, but it

would appear, as Hutchinson states, that the carpel is universally accepted by taxonomic writers as a modified leaf, and the same may be said of the stamens and other organs.

In view of the suggestion that the present writer would have done better had he joined in the study of ontogenetic development, and from other remarks, it may be that our critic places special reliance on his observations in this field. But again it has yet to be shown that the ontogenetic study of floral parts is a certain guide to their evolutionary history.

On the contrary, it seems clear that students of floral ontogeny may reach conclusions about the origin of the carpel and stigma which are as varied as those reached by other modes of approach. For example, two interesting papers (Eber¹(2), Hagerup⁽⁵⁾) have been published during the present year in which the authors suggest views very dissimilar to those of Prof. Thompson from their developmental studies. Thus we may still say with Goebel (3), p. 9) that "obviously even developmental history is not, as a method, the whole and sole 'way of salvation'. For it is not the facts thereby attained, but the conclusions drawn from them that determine the progress of science. These conclusions are influenced not only by each particular investigator's individuality but by the general posture of science in his day."

It follows then that before we can evaluate any morphological generalisation we must know the premises which were present in the mind of its author. Prof. Thompson has studied developing flowers. Why does he believe that his observations entitle him to conclude that stigmas have been evolved from sterile stamens? No doubt he has reasons, but he can scarcely blame botanists (cf. Parkin⁽⁷⁾) for regarding his views as entirely speculative until they know what these reasons are. We should like to be able to take these views into serious consideration but their author must give us more information. He must tell us exactly what he means by "stigmatism", and "the view that stigmatism may declare itself first at the apex of the style"? Can the statement that stigmatism "expresses a state of tissue in some way associated with limitation of growth" be expounded in terms of plant physiology or physiological anatomy? He points out that consideration of the processes within and without the cupule-like envelope of *Caytonia* is for ever debarred, but he gives us no

¹ Many of the interesting facts set out by Miss Eber, such as those concerning the development of the stigmas of *Zostera maritima*, seem capable of interpretation on the lines of my hypothesis.

indication of the methods or extent of his consideration of the processes taking place in the stigmas of the Scitaminean plants during their development. In fact, he gives us no information about his material in its living state; there are no data about the rate of growth or state of nutrition of the receptacle, the periods of time occupied in development, or the conditions under which his flowers were produced.

The criticism that there is no evidence for my conclusion that the stigma originated on the ventral side of the carpel raises another aspect of the methods of morphologists. What is evidence for any such conclusion, and how is it obtained? The method is as follows. A number of plant structures or facts of development are observed: they may be regarded as objective facts. These facts are then arranged in some definite order [cf. Crow(1)], the order chosen is generally, if not always, the outcome of some previous knowledge or supposition. By a comprehensive view of the facts as so arranged, a morphological generalisation is reached. So that the evidence for a theory is, first that certain facts can be observed, and secondly that they can be arranged in a particular way. In my paper information collected by myself and others about stigmas and transmitting tissue was arranged in a certain order and a conclusion was reached.

But the ascertainable facts about Angiosperm flowers have been arranged in many other ways by different authors who have reached conflicting conclusions. The problem then is to find the correct mode of arrangement. We need a clue to *this* problem, and in the writer's opinion the clue may be found by studying fossil plants. The discovery of fossil Angiosperms can never by itself solve the problems of floral structures, but it may nevertheless provide the indications by which all the known facts of the structure and development of living plants may be fitted into series which approximate to the true phyletic series.

The next point concerns the status of the concept reached by any arrangement of observed facts. Prof. Thompson suggests that my ideas on the stigma are entirely subjective although I professed to aim at an objective approach; on the other hand he considers his view to offer "the first objective statement on stylar origin". Now when we have observed a series of facts, and have grouped them, we may reach, as the result of a mental process, a concept which seems to us to explain the relationship of the facts. We cannot create an actual plant which performs the supposed evolutionary process, and consequently the suggested course of evolution takes place only in our

minds. For this reason the concepts of morphologists have often been termed subjective, and some authors have suggested that they can never be otherwise. It may be that this is so, but it need not deter us from constantly reminding ourselves, and others, of the need of trying to avoid concepts which are based solely on a mental picture.

Supposing that we have reached a view of the possible evolution of the stigma as the result of comparing actual plants. The first thing to be asked is whether the suggested scheme is physiologically possible. If we are satisfied that a hypothetical process could actually operate, it may be possible to advance further in our quest for objectivity. We may ask—is there evidence that plants or plant organs have existed in the past which possessed structures of the type we have imagined as primitive in our evolutionary scheme? If such structures have once existed, it matters little that we cannot prove their affinities or their direct connection with groups we have been studying. The structures pictured in the mind become of higher scientific significance.

Now we may also be able to find series of extinct plants. If these are arranged in the order of their appearance the conclusions drawn from them will have a high grade of reliability, since the series can only be read in one chronological order. But if such a series is taken by itself the concepts reached are still subjective, since we can never be sure that the successive structures possess a lineal relationship.

Our critic will welcome the testimony of authentic Angiosperms from the rocks, while discounting any evidence provided by the Caytoniales. He must tell us, however, how he would recognise an authentic angiosperm of a very primitive type, and why we must set aside the *actual* record of fossils, scanty though it is.

In the physical sciences the validity of conclusions or hypotheses can be tested by controlled experiments or by the comparison of observed results with a theoretical equation. Such methods involve processes of correlation. It is to correlations that the morphologist must turn in order to find out whether his subjective conclusions can be reasonably regarded as representing actual facts. The test of a generalisation must be the nearness with which it explains and correlates *all* the available series of observations.

Now we may compare modern carpels from eight or more different aspects. We can arrange our observations in a serial form or in several different forms as the result of studies from each aspect, and from every series reach a view of carpel evolution. Thus the study of the vascular system may lead to views that the earliest carpellary

structures were of the nature of simple infolded leaves or peltate leaves, or that they were compound or trilobed leaves, or that structures formerly interpreted as simple leaves originated from two or three distinct organs (the polymorphic view), or that the earlier structures were a pair of ovules surrounded by cupules on a short axis, or that the ancestors of the carpel-bearing plants were acarpous. In just the same way we may arrive at several divergent or contradictory views by the examination of the form of stigmas and of the transmitting tissue, or by developmental studies. Thus the investigation of carpels from every different aspect might result in a large number of separate assessments of the nature of the primitive carpel. Now if the ontogeny of the carpel, its vascular system, its stigma forms, its abnormalities, etc. are independently variable—a point needing further debate—is it not most likely that the real ancestral carpel type was that common form which is surmised by the convergence of the greatest possible number of lines of independent argument? If only some statistical method like that employed by wood anatomists [cf. Frost (2a)] could be devised for demonstrating correlations, and the personal advocacy or declamatory denial of different views replaced by a mathematical process, considerable service would be rendered to theoretical morphology. But as the matter stands at present, the combination and correlation of the evidence from every possible source seems to provide the surest way of reaching reliable conclusions on the evolution of the carpel, conclusions which are the least influenced by the observer's individuality.

The hypothesis which I put forward in my previous paper was not a mere attempt to magnify the importance of the Caytoniales but was reached after a consideration of the evidence from all sources. It would not be invalidated by the complete withdrawal of fossil evidence. In a short article it was not possible to discuss every point in great detail, but my critic is scarcely correct when he says that the major portion of the writer's essentials for a morphological system had been forgotten in the discussion. Readers are invited to verify this, and to form their own opinion as to the alleged fatal omission of the stamens and perianth from the discussion of the stigma. Is it reasonably certain that all the earliest angiospermous flowers were bisexual?

Attempts to find a single hypothesis which provides a means of connecting together all the facts ascertainable by independent lines of inquiry can also be made in dealing with subsidiary matters such as the problem of the status of *Drimys*. A comparison of the statements

made by me and by my critic about the stigmas of this and its allied forms illustrates very well the lines of approach of their respective authors. I gave a short account of the objects on which, among others, was founded the view that the stigma of simple carpels is a double lateral structure, the view which formed the real foundation of my paper; Prof. Thompson, forgetting perhaps that this view preceded the discovery of the Caytoniales by seventy years, regards this account as a distraction. What Robert Brown described as "two stigmata which are to be regarded not as terminal but lateral", is termed by my critic an undivided and U-shaped terminal, and it is implied that such a structure furnishes little support for the writer's contentions. But the pertinent question is whether the carpels and stigmas of *Drimys* are to be regarded as primitive or advanced in type. Prof. Thompson writes of one of the figured forms as advanced, without giving any reason for his opinion. I, however, thought that a good case could be made out for regarding *Drimys* as an archaic type, whose carpels should be carefully studied. There are four quite independent lines of argument for this view. (1) The structure of the wood, especially in view of the recent investigations of Frost (2a), Sahni (8) and Gupta (4); (2) the geographical distribution of the genus; (3) the fossil record of the group and (4) the general construction of the flower. Not one of these arguments considered by itself could be regarded as decisive, and yet taken together they make it somewhat difficult for us to regard *Drimys* as an advanced type.

It is a matter for regret that I apparently failed to present a clear picture of some of the concepts put forward in "the Theory of Scitaminean flowering" and of their evidence. Botanists who have read the original paper, my remarks and Prof. Thompson's rejoinder, must apportion the blame. But my remarks on this publication have had a useful result. They have given Prof. Thompson the opportunity of correcting the false impression that the form and number of the floral parts did not depend on heredity, and also of making clear that the numbering of the primordia in, say, the flower of *Musa* was the result of actual observation of the growing flower. May one express the hope that he will later provide illustrations of his actual preparations in further demonstration of this most interesting point?

But when all has been said for and against our new theories, the fact remains that botanists have got to face the problem of so adjusting their morphological principles as to make them consonant with modern knowledge. The so-called New Morphology is an attempt

to do this. If those whose work is centred on living plants think the attempt futile, they should try to produce something which will take its place. Prof. Thompson's ideas do not agree very well with our classical morphological system and it will be interesting to learn what modifications he proposes to make. He "confesses to a return to a view of flowering not far removed from that of Schleiden of a hundred years ago, though it may be dressed in different clothes". He commenced his criticism with a quotation from Schleiden in order to point a moral. May one remind readers that in the *Principles of Scientific Botany* (9) this quotation precedes by a few pages the vigorous assertion by its writer that the plant embryo was formed from and developed inside the tip of the pollen tube? This view led to the concept that there is not even a remote analogy between the anther and the antheridium (p. 359), and that "the anther of the plant is nothing else than a female . . . ovary while each pollen grain is the germ of a new individual" (Goebel (3), p. 20). Schleiden scorned what he described as "the trifling, though very pretentiously delivered, new researches of Amici" (9), p. 413), which were, however, shortly afterwards fully confirmed by von Mohl, Hofmeister and others. Since Schleiden's interpretation of the floral organs centred on such an erroneous idea, a return to his concepts cannot fail to be interesting.

But though Schleiden's view of reproduction, like his view of cell formation, was completely mistaken, yet his work, with its many aspersions on the intellect and veracity of his contemporaries, had a great influence on the development of Botany, especially in Germany. This was mainly because it stimulated thought and discussion. Young botanists tried to find out the truth for themselves. It is very probable that the views of the present writer and of his critic will eventually both prove to be mistaken. But they will not be useless if they lead to discussion and further investigation. The physiological aspect of the stigma and the transmitting tissue is as yet almost untouched, though enough has been done to show that it will not be an easy subject of research. Surely the attitude of the scientist should not be to "wait with patience the story of living angiosperms", but to examine critically the ideas and arguments put forward by each writer, and to check them by reference to old and new observations.

Plant Morphology is often regarded as a subject of minor scientific importance, being either purely descriptive or mainly speculative. This view is perhaps true with reference to work on the higher plants, but it may well be the fault of the workers rather than of the subject. There is no fundamental difference

between attempts to correlate and explain observed facts in morphology and those in physiology, genetics or other branches of science. The concept of the atom was a subjective speculation on the part of chemists, which is undergoing considerable modification as the result of modern physical research, but it acquired importance because it affords a means of correlating so many observations on chemical reactions. I believe that we are now gradually reaching conclusions which will enable us to correlate many of the structural features seen in the Pteridophyta. But little real progress has been made from work on the seed plants, and this may be attributed to our failure to base our arguments on sure foundations. Most writers cling to the dogmas of the early morphologists and try to build on some of the pre-Darwinian concepts of plant form. So papers are published containing generalisations which are purely speculative or appear so to those who are ignorant of the pre-conceptions present in the mind of their author. Even when we abandon the classical morphology a similar situation may arise as shown by the present discussion. If our studies are to help in the advancement of our science I feel sure that we must examine most critically our basic ideas, our mode of reasoning and our methods of work, we must try to state the foundations of our views and take nothing for granted. Constructive criticism is also very valuable, and for this reason I hope that this exchange of views with Professor McLean Thompson may have some permanent influence in promoting the progress of our subject.

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A CRITICAL REVISION OF CERTAIN TAXONOMIC GROUPS OF THE MALVALES

PART II¹

By H. L. EDLIN, B.Sc.

(With 1 figure in the text)

DELIMITATION OF THE ORDER MALVALES

THE order Malvales may be diagnosed as: Dicotyledons, polypetalous, hypogynous, with axile placentation and valvate sepals. The prefloration of the sepals separates the order from the Guttiferae, Theaceae, and allied families; the placentation excludes from the order such genera as *Nettoa*, and includes within it genera such as *Prockia* and *Hasseltia* (excluded by Engler and Prantl(2)).

The general characteristics are: trees, shrubs or herbs; bark fibrous; sap viscid; indumentum stellate or lepidote. Leaves stipulate, alternate, often palmately nerved, simple, lobed or digitate. Inflorescence cymose, often axillary. Flowers actinomorphic, often large and showy, bisexual (rarely unisexual); epicalyx or bracteoles often present. Calyx valvate. Corolla imbricate or contorted. Stamens numerous, free, polyadelphous, or monadelphous; with or without staminodes; anthers often reniform and unilocular, or bilocular, rarely compound. Carpels united, often numerous; placentation axile; styles simple or branched. Fruit capsular, baccate, apocarpous, or schizocarpous. Seeds generally few per carpel; or numerous, in which case often clothed with fibrous hairs; endosperm present or not; embryo variable. The most marked characteristic of the wood anatomy is the occurrence of "tile-cells" in certain genera; these cells have not been noted in any other order of plants.

It will be seen that the Malvales are well defined by characters other than purely floristic ones; they form a very natural order. Only within the order has serious difference of opinion arisen as regards classification. It may be argued that the so-called families of the Malvales, are not clear-cut families, as the term is generally applied, but are only representative of vague evolutionary trends. But in the following pages it will be shown that all the genera may be assigned to families which are separated from each other by clearly marked distinctions.

¹ Part I appeared in *New Phytol.* 34, 1.

DELIMITATION OF FAMILIES

After examining material of all available genera, and considering the descriptions of these and others given by Bentham and Hooker(1), Engler and Prantl(2), and other writers, it appears that all the genera of the Malvales may be grouped into six families, as follows in the succeeding pages. The names of the tribes are mainly those adopted by Engler and Prantl; but where alterations are proposed, the names and the composition of the tribes have been changed. Numbers cited for the genera and species of a family are only approximately correct; they are based, with occasional alterations, on Willis's *Dictionary*(7).

FAMILY SCYTOPETALACEAE

Arborescent Malvales without an epicalyx, with a gamosepalous calyx, and 3 to many petals. Stamens 8 to numerous, free, in irregular clusters; anthers bilocular. Ovary 3- to 6-locular; style bent; stigma small; ovules pendulous; fruit woody. Leaves simple, penninerved, exstipulate. Petals linear, rarely absent.

Genera

Scytopetalum Pierre
Brazzeia Baill
Rhaptopetalum Oliv.
Oubanguia Baill

Pseudobrazzeia Engl.
Gonystylus Teijsm. et Binn.
Erythropyxis Pierre
Solmsia Baill.

As thus constituted, this family consists of trees only, and is confined to Tropical West Africa and Malaya. No known economic importance. 8 genera, 33 species.

FAMILY TILIACEAE

Malvales with numerous stamens (rarely 10 or fewer), free or very shortly connate into a tube or phalanges. Anthers bilocular. Leaves stipulate.

Genera

ELAEOCARPEAE
Elaeocarpus L.
Sloanea L.
Crimodendron Molina
Dubouzetia Panch.
Antholoma Labill
Muntingia L.
Anonodes Schltr.
Seruclea Schltr.

Aceratum Schltr.
 ARISTOTELIEAE
Vallia Mutis
Aristotelia L'Herit.
 PROCKIEAE
Prockia L.
Hasseltia H.B.K.
Plagiopteron Griff.

BROWNLIEWIAE

Carpodiptera Gris.
Berrya Roxb.
Christiana DC.
Chartocalyx Mast.
Brownlowia Roxb.
Pentace Hassk.
Diplodiscus Turcz.
Pityranthe Thw.
Astrophorum Sprague
Tahitia Burr.

APEIBEAE

Ancistrocarpus Oliv.
Glyphaea Hook. f.
Apeiba Aubl.

TILIAE

Entelea R. Br.
Corchorus L.
Sparmannia L. f.
Honckenia Willd.
Luehea Willd.
Mollia Mart.
Graeffea Seem.
Trichospermum Bl.

Schoutenia Korth.

Tilia L.
Vasivaea Baill.
Luheopsis Burr.
Ceratosepalum Oliv.
Cephalonema K. Schum.
Tetralix Griseb.
Sicrea Hallier.

GREWIEAE

Grewia L.
Duboscia Bocq.
Desplatia Bocq.
Diplophractum Desf.
Columbia Pers.
Belotia A. Rich.
Ermocarpus Nimmo
Heliocarpus L.
Althoffia K. Schum.
Ledermannia Mildbr. et Burr.
Goethalsia Pittier
Cotylonychia Stapf
Eleutherostylis Burr.
Grewiopsis De Wild. et Dur.
Halconia Merrill
Triumfetta L.

Herbs are uncommon in this family which, with the exception of *Tilia* in the northern hemisphere, is confined to the tropics. *Corchorus* (jute), *Grewia*, *Triumfetta*, *Tilia*, and other genera, yield fibres. *Tilia* also gives timber, and is planted for ornament. 70 genera, 540 species.

FAMILY STERCULIACEAE (emac.)

Arborescent Malvales with unisexual apetalous flowers; anthers bilocular; no staminodes. No epicalyx; calyx gamosepalous, generally coloured. Ripe carpels separate in fruit. Leaves stipulate.

Genera

STERCULINEAE

Sterculia L.
Cola Schott.
Tetradia R. Br.
Octolobus Welw.

TARRIETIEAE

Tarrietia Bl.
Heritiera Ait.
Argyrodendron F.v. Muell.

This family is entirely arborescent and tropical. Many species growing in rain forests are remarkable for their development of plank buttresses. Species of *Cola* yield kola nuts; most of the genera give good timber, that of *Tarrietia* and *Heritiera* being of economic importance; species of *Sterculia* and *Brachychiton* are planted for ornament. 7 genera, 175 species.

FAMILY BUETTNERIACEAE

Malvales with 5 to numerous stamens, arranged singly, or in groups, alternating in a definite arrangement with petaloid staminodes. Exceptionally, staminodes absent, in which case stamens 5 (and) or monadelphous. Anthers bilocular. Leaves stipulate. Flowers always bisexual.

Genera

ERIOLEAENEAE

Eriolaena DC.

DOMBEYEAE

Melhania Forsk.

Pentapetes L.

Trochetia DC.

Cheirolaena Benth

Dombeya Cav

Ruzia Cav.

Astria Lindl.

Corchoropsis Sieb. et Zucc.

Paradombeya Stapf

Humbertiella Hochr

Harmsta K. Schum.

HERMANNIAEAE

Hermannia L.

Melochia L.

Dicarpidium F v M.

Waltheria L.

Physodium Presl

BUETTNERIACEAE-BUETTNERINAE

Rulingia R Br

Commersonia Forst.

Buettneria L.

Ayenia L

Craigia W W. Sm.

Nephropetalum Robinson et

Greenman

BUETTNERIACEAE-THEOBROMINAE

Glossostemon Desf.

Scaphopetalum Mast.

Leptonychia Turcz.

Abroma L.f.

Theobroma L.

Guazuma Plum.

Leptonychopsis Ridl

Peniculisera Ridl.

Herrania Goud.

LASIOPETALEAE

Pimia Seem.

Hannafordia F v M

Thomasia Gay

Guichenotia Gay

Keraudrenia Gay

Seringea Gay

Lysiosepalum F.v.M.

Lasiopetalum Sm.

Hymenocapsa J. M Black

HELICTEREAE

Pterospermum Schreb.

Helicteres L.

Kleinovia L.

Reevesia Lindl

Ungeria Schott et Endl.

MANSONIEAE

Mansonia J R Drumm

Triplochiton K. Schum.

Cistanthera K Schum.

A tropical family of herbs, shrubs and trees. *Theobroma* is cultivated in Africa and America for its fruits, from which cocoa is prepared. *Mansonia*, *Triplochiton*, and *Cistanthera* are important West African timber trees. 49 genera, 610 species.

FAMILY BOMBACACEAE

Malvales with numerous unilocular (rarely compound) anthers, on filaments united in a tube or phalanges, or in both. Fruit of 2 to 10

carpels, loculicidally dehiscent (capsular), or baccate. Leaves stipulate.

Genera

ADANSONIEAE

Adansonia L.
Bombax L.
Pachira Aubl.
Chorisia H.B.K.
Ceiba Gaertn.
Neobuchia Urb.
Bombacopsis Pittier
Cutostemma Benth.
Bernoulia Oliv.

MATISIEAE

Cavanillesia Ruiz. et Pav.
Hampea Schlecht.
Scleronema Benth.
Matisia Humb. et Bpl.
Montezuma Moc. et Sess.
Quararibea Aubl.
Ochroma Sw.

DURIONEAE

Cumingia Vidal
Camptostemon Mast.
Durio L.
Neesia Blume
Boschia Korth.
Coelostegia Benth.
Cullenia Wight

HIBISCEAE

Decaschistia Wight et Arn.

Senra Cav.

Lagunaria G. Don
Hibiscus L.
Abelmoschus Medic.
Kosteletzkya Presl
Dicellostyles Benth.
Julostyles Thw.
Thespesia Soland.
Shantzia Lewton
Cienfuegosia Cav.
Gossypium L.
Ingenhousia Moc. et Sess.
Pseudopavonia Hassl.
Maga Urb.
Kokia Lewton
Hibiscadelphus Rock
Wercklea Pitt. et Standl.
Megistostegium Hochr.
Erioxylum Rose et Standl.
Cenocentrum Gagnepain
Symphyochlamys Gurke
Howittia F.v.M.

KYDIEAE

Kydia Roxb.

FREMONTIEAE

Fremontia Torr.
Cheirostemon Humb. et Bpl.

Mainly trees and shrubs, confined to the tropics and subtropics. *Ceiba* (Kapok), *Bombax* (Semul), *Gossypium* (Cotton), yield fibres of the first economic importance. The wood of *Bombax* and *Ochroma* (Balsa) is valued for its lightness. *Adansonia* and *Durio* have edible fruits, and *Hibiscus* sp. and *Fremontia* are garden shrubs. 48 genera, 485 species.

FAMILY MALVACEAE

Malvales with 5 to many unilocular reniform anthers on monadelphous filaments. Leaves stipulate, palmi-nerved, entire or lobed. Style divided. Fruit of 5 to many (1-2 in New Zealand genus *Plagianthus*), septicidally dehiscent schizocarps. Epicalyx often present, sepals 5, petals 5, conspicuous.

Genera

MALOPEAE

Malope L.
Kitaibelia Willd.
Palava Cav.

MALVEAE-ABUTILINAE

Abutilon Gaertn.
Wissadula Med.
Sphaeralcea St Hil.
Modiola Moench
Horsfordia A. Gray
Bakeridesia Hochr.
Pseudabutilon R.E. Fries
Abutilothamnus Ulbrich.
Modiolastrum K. Schum
Neobrittonia Hochr.

MALVEAE-MALVINAE

Lavatera L.
Althaea L.
Malva L.
Sidalcea A. Gray
Napaea L.
Malcastrum A. Gray

MALVEAE-SIDINAE

Plagianthus Foist
Sida L.

Gaya H.B.K.

Anoda Cav.
Hoheria Cunningham
Cristaria Cav.
Halothamnus F.v.M.
Peviptera DC.
Nototriche Turcz.
Bastardiopsis Hassl.
Tetraptera Phil.
Lawrencia Hook. f
Pseudobastardia Hassl.
Briquetia Hochr.
Tarasa Phil.
Robinsonella Rose et Baker f.
Bastardia H.B.K.

URENEAE

Malachra L.
Urena L.
Pavonia L.
Goethea Nees et Mart.
Malnaviscus Dill
Lopimia Mart
Malache Vogel
Blanchetiastrum Hassl.
Peltaea Standl

A cosmopolitan family, herbaceous or only softly woody (*Hoheria*). A few are cultivated for fodder or fibre. *Malope*, *Abutilon*, *Lavatera*, *Althaea*, *Malva*, *Sida*, and others, are of value in horticulture. 45 genera, 550 species.

KEY TO THE FAMILIES OF THE MALVALES

- a Anthers bilocular, stamens not adnate
 - b Leaves exstipulate, stamens indefinite, calyx gamosepalous, trees
Scytopetalaceae
 - b. Leaves stipulate.
 - c. Fruit apocarpous (ovary syncarpous); flowers apetalous, unisexual, staminodes absent, trees
Sterculiaceae
 - c Fruit syncarpous, type variable
 - d. Stamens numerous, free, irregular (in relation to the staminodes)
Tiliaceae
 - d. Stamens definite, or united, or in regular alternation with petaloid staminodes
Buettneriaceae
- a. Anthers unilocular, filaments connate and adnate to the petals at the base.
 - e. Fruit loculicidally dehiscent, or indehiscent, capsular or baccate; carpels 2 to 10, mainly trees or shrubs
Bombacaceae
 - e. Fruit septicidally dehiscent into many (rarely few), usually few-seeded, schizocarps; mainly herbs with conspicuous flowers
Malvaceae

CONSIDERATION OF THE REARRANGEMENT

The chief advantage of this proposed rearrangement of genera is that it refers every genus to a definite clear-cut family; this, in itself, is enough to outweigh the slight alteration from previous classifications. It seems better to refer atypical genera, such as *Kydia*, to definite families for definite reasons, than to place them in ill-defined families on account of vague resemblances or affinities.

The key characteristics adopted for the families are usually those which are the essential peculiarity of the family, and which afford a clue to its phyletic position. Thus, the apocarpous fruit is the outstanding peculiarity of the Sterculiaceae; and the few, united, or regular stamens mark out the Buettneriaceae as more advanced than the Tiliaceae. The unilocular anther sets the Bombacaceae and Malvaceae apart as a particular group; and the schizocarpous fruit is, above all, the most striking peculiarity of the Malvaceae.

The chief changes in arrangement here proposed are: the composition of the family Sterculiaceae has been altered, so that it comprises only the tribe Sterculieae; the tribe Hibisceae has been transferred from the Malvaceae to the Bombacaceae; three other genera of the Malvaceae have been removed to the Bombacaceae.

The disadvantages of this arrangement are particular rather than general. The line of distinction between the Tiliaceae and the Buettneriaceae is purely arbitrary; but it is definable, and the close affinity between the two families is brought out by the key.

This rearrangement affects the relative standing of the Malvaceae and Bombacaceae. The former, as here recognized, is a small and highly specialized family, and the latter a large and more varied one. But this is in accord with their evolutionary position, since the Bombacaceae are a transitional, and the Malvaceae a climax, family. The conception of both, as natural families, is thus strengthened.

The following suggested subdivision of the family Bombacaceae, as here delimited, shows that it is not artificial in its composition.

- | | |
|--|--------------------|
| a. Anthers at apex only of the staminal column or filaments. | |
| b. Flowers without epicalyx or bracteoles. | |
| c. Leaves digitately compound | Adansonieae |
| c. Leaves simple | Matisieae |
| b. Epicalyx or bracteoles present. | |
| d. Nervation of leaves pinnate | Durioneae |

- d. Nervation of leaves palmate.
- e. Flowers apetalous **Fremontieae**
- e. Flowers petaliferous, bracteoles enlarging below the fruit **Kydiseae**
- a. Staminal column antheriferous below the apex **Hibisceae**

PHYLOGENY OF THE MALVALES

As the main theme for discussion here is not the external relationships of the Malvales with allied orders of plants, it will be sufficient to state the views of Warming (6) and Hutchinson (5), which are held by most other writers. They agree that the Malvales are more advanced than, and possibly derived from, the Dilleniaceae; and that they are less advanced than, and possibly ancestors of, the Euphorbiaceae.

The first point must be to determine which families or tribes of the Malvales are relatively primitive, and which relatively advanced. Warming regards the Sterculiaceae, with few stamens, as primitive; the Tiliaceae, with many stamens, as intermediate; and the Malvaceae, with many united stamens and unilocular anthers, as advanced. Hutchinson describes the Malvaceae as "A very natural group representing a fixed type of the Tiliales, and whence little or no further evolution has proceeded".

The next consideration is the evidence afforded by taxonomy. It is logical to argue that the most primitive members of a group are those which most resemble the group before it in sequence—in this case, the Dilleniaceae. The Tiliaceae and Scytopetalaceae resemble this family in the androecium; for the stamens are numerous, hypogynous, and free or variously united into bundles at the base. The Sterculiaceae resemble the Dilleniaceae in the gynoecium, for this is apocarpous, often with many carpels.

Similarly, it may be expected that the most advanced members of the group will simulate the one next after it, that is, the Euphorbiaceae. The characteristic feature of this family, the trilocular ovary, is found in *Kydia*, *Howittia*, and certain Hibisceae (e.g. *Gossypium*). The fruit of the sand-box tree (*Hura crepitans* L., Euphorbiaceae) which is schizocarpous in structure, presents a striking analogy with the typical fruit of the Malvaceae. Thus, a consideration of the external relations of the group, suggests that the Scytopetalaceae, Tiliaceae, and Sterculiaceae are primitive, and the Malvaceae advanced.

If a group of plants manifests a peculiar characteristic, then that

characteristic will be intensified in the advanced members of the group; this is especially the case in one which forms an evolutionary blind-alley, and does not lead on to a higher group. One such characteristic of the Malvales is the valvate calyx, which is scarcely obvious in the Scytopetalaceae and in *Gonystylus*, but reaches its highest development in the Malvaceae proper. Another is the stellate indumentum, which is best shown by the Malvaceae; many of the Bombacaceae show an advanced modification of it in the shape of lepidote scales. A third is the reniform unilocular anther, which feature is confined to the Malvaceae and Bombacaceae. Finally, the multilocular schizocarpous gynoecium is only found in the true Malvaceae, and reaches its highest modification in the Malopeae, where the schizocarps are one-seeded and in several whorls. On this line of argument, then, the Malvaceae are the most advanced family.

The wood structure of the Tiliaceae points to this family being primitive, at least more primitive than the Sterculiaceae and the Buettneriaceae.

Genera which have been referred, not only to the order Malvales, but also to other orders (such as *Prockia*, sometimes placed in the Bixales; and *Pentadiplandra*, also put in the Celastrales) have almost invariably been assigned to the Tiliaceae. Therefore, that family must occupy a marginal position in the order, either at the upper or lower limit of evolution. ✓

The principles generally followed in deciding whether a plant structure is primitive or advanced may next be considered. Trees and shrubs are generally more primitive than herbs: from which it follows that the Malvaceae (as here delimited) constitute the most advanced group. Free petals are more primitive than connate petals; the Malvaceae and Bombacaceae, with petals connate at the base, are, therefore, advanced. The same conclusion is reached on considering the staminal insertion; these two families are slightly perigynous (markedly so in *Ochroma*, Bombacaceae), and therefore are more advanced than the other hypogynous families. Separate stamens precede connate stamens; hence, the Scytopetalaceae and Tiliaceae are primitive; the Sterculiaceae, Buettneriaceae, and Bombacaceae are intermediate; and the Hibisceae and especially the Malveae (stamens connate to the summit of the column) are advanced.

Hutchinson (5) stresses the point that many-parted (polymerous) flowers are primitive, and states that "in the primitive flowers there are many stamens, in the higher flowers few stamens;" he speaks of a "progressive sterilisation of reproductive parts (sporophylls)", which

accompanies evolution. On these grounds, the Buettneriaceae and their allies, especially the Hermannieae (e.g. *Waltheria* with 5 stamens) and Lasiopetaleae, are more highly evolved than the Tiliaceae.

Free and numerous carpels are considered primitive. The Sterculiaceae (especially *Octolobus* with more than 60 free carpels) are therefore very primitive in this respect. The same cannot be said of the Malvaceae, in which the carpels separate in the fruit, for there are grounds for supposing that this structure must have arisen from a *united* gynoecium; further, many Malvaceae (e.g. Ureneae) have only five carpels.

Summing up this evidence, it may be said that the Scytopetalaceae and Tiliaceae are primitive families, the Buettneriaceae more advanced than these, the Bombacaceae highly evolved, and the Malvaceae the most highly evolved of the order. The position of the Sterculiaceae is doubtful; the structure of their gynoecium is primitive, that of the rest of the flower is advanced but along lines not found elsewhere in the group.

The general anatomy and wood structure of the Malvales appear to be more remarkable for homogeneity than for marked differences between the families; but the absence of tile-cells from the wood structure of the Sterculiaceae, lends weight to the view that this is a primitive family.

CHORISIS

Warming's (6) sequence of the families differs from that arrived at above, inasmuch as the Buettneriaceae, with few stamens, are considered more primitive than the Tiliaceae, which have many. This conclusion follows from an application of the theory of chorisis to the group; as was made by Asa Gray (1).

According to Goebel (3), the term "chorisis" (Fr. *dédoublement*; Ger. *Verdoppelung*) was first used by Dunal, and has been defined by Moquin-Tandon as follows. "When in the place of one stamen, which ordinarily exists in an organic symmetry, one finds many stamens, these have become many by *dédoublement* or multiplication." In criticism of this Goebel states: "Have we now a right to make such an assumption... It is clear that only the history of development and the comparison with allied forms can give information as to the actual process. In many cases, as is shown by the whole configuration of the flowers concerned (e.g. *Papaveraceae*), the contention is quite untenable." He then considers the special case of *Hypericum aegyptiacum* in which a bundle of stamens has been recognised (by Warming

and others) as one branching leaf, and states: "Against this I have already shown that the comparison of the different forms of flower and their development makes possible another suggestion, namely, derivation from a flower which forms numerous stamens in descending serial succession uniformly distributed on the torus."

It is significant that the family Hypericaceae is closely allied to the Malvales. In a later edition of the same work, Goebel declares: "In the acyclic flowers, to which the chorisis theory has not been applied, no one doubts that the polyandrous flowers are primitive. On what account can it be otherwise in the cyclic flowers?" Concerning the chorisis of stamens, he states: "It is indeed possible that in many cases a complete splitting of the staminal primordia takes place, and there are certain constant examples of an incomplete splitting, e.g. Malvaceae; we know of cases here, where each single stamen splits into halves, each bearing a unilocular anther."

Of chorisis of the carpels, Goebel states: "The number of carpels may also increase by branching, for example, in many Malvaceae. Payer found in *Kitaibelia vitifolia* five carpellary primordia, out of which by branching and the formation of false septa, numerous monospermous ovaries are developed. In *Malva* and others, the numerous carpels appear to be separated from the first. The process is in any case a rare one, and is undoubtedly connected here with the formation of the monospermous schizocarps in place of the capsule." *Kitaibelia* is a genus of the Malopeae, which have carpels clustered in a head in several whorls.

How far, then, may one accept or reject chorisis as explaining the phylogeny of the Malvales? It is clearly a logical explanation of the unilocular anther, but this feature is confined to the Bombacaceae and the Malvaceae, which are generally agreed to be more advanced than the Tiliaceae. In *Bombax malabaricum*, individual stamens may be found with the filaments of the unilocular anthers united in pairs right to the tip, or divided at any distance down, almost to the base. Thus one bilocular stamen, may become, by chorisis, two unilocular stamens. These may, in turn, become four stamens each with a single pollen sac, though this state has never been noted amongst the Malvales. But this is the limit beyond which the process cannot proceed. It accounts very well for the existence of numerous unilocular stamens in the place of half as many bilocular stamens; to call upon it to account for the existence of numerous stamens (either unilocular or bilocular) in place of only five or ten stamens is a *reductio ad absurdum*.

As an explanation of the numerous carpels of the Malvaceae, chorisis fits the facts very well; further, it is, in part, proven. The occurrence of genera with only 5 carpels, and the fact that the multitudinous carpels of the Malopeae are in five vertical ranks, clearly points to a splitting of five primordia. It may hardly be extended to the Sterculiaceae, in which the numerous carpels are almost apocarpous, and each appears to be of separate origin (e.g. in male flowers of *Octolobus*).

In the light of these opinions, Warming's sequence of the families appears to be unsound. His theory may be briefly stated thus: the stamens of the Buettneriaceae become multiplied by chorisis, and so we get the Tiliaceae; the stamens then become connate, and we arrive at the Malvaceae. The flaws in the reasoning are: in many of the Buettneriaceae (e.g. *Buettneria*) the stamens are connate, hence they are more advanced than the Tiliaceae; further, staminodes occur, pointing to a reduction from some polystemonous type; chorisis cannot well be applied to the androecial structure of the Tiliaceae, but only to that of the Malvaceae. The theory, therefore, must be rejected, and an alternative one advanced.

A PHYLETIC HYPOTHESIS

The best clues to the phylogeny of the Malvales are afforded by the varying construction of the androecium and the gynoecium. These two floral whorls exhibit a sufficient range of variation; for free, polyadelphous, and monadelphous androecia occur, in addition to various types of staminodes; and the gynoecia may be apocarpous, gamocarpous, or schizocarpous. Other characteristics which might be used are too constant throughout the group; as: the stellate pubescence, the valvate calyx, and the imbricate or contorted corolla. Certain characteristics, useful elsewhere, are too variable within closely allied groups; as: the leaf venation, the presence of an epicalyx, the cohesion of the sepals, the type of inflorescence, apetaly, and unisexuality. Still other characters must be rejected owing to their difficulty of determination, in some members at least of the group; these include the dehiscence of the anther, the nature of the pollen, and the presence or absence of endosperm and mucilage canals.

The androecium is the most primitive in the Scytopetalaceae. In *Scytopetalum tieghemii* Hutch. et Dalz., there are very numerous stamens, slightly united in clusters at their bases, these clusters being arranged in several whorls or series on the disc, some being

antipetalous. They thus approximate to the multiseriate stamens of the Magnoliaceae.

The Tiliaceae, with many stamens either free or very shortly united in antipetalous clusters, in a single whorl or series, are more advanced. To account for this evolution, the following theory is advanced: In primitive flowers such as the Magnoliaceae, the stamiferous area forms a ring near the top of the torus; in more advanced flowers, such as *Scytopetalum*, the stamiferous area is confined to isolated patches in several series within this broad ring; in the majority of flowers this ring is reduced to a circle, or a pair of concentric circles, which, in many forms, are only stamiferous at certain fixed points. Where two such circles occur, it is *usual* for the stamiferous points on the outer circle to alternate with the insertion of the petals; the stamiferous points on the inner circle likewise alternate with those on the outer; thus it happens that the outer stamens are antisepalous, and the inner stamens antipetalous. Exceptionally, as in some Malvales, the position of the staminal whorls in relation to the perianth whorls is reversed, and we arrive at the obdiplostemonous condition.

This argument proceeds from a polyandrous androecium, and works towards a pentamerous one; but few will doubt that this is the natural sequence of evolution. A survey of all the families of the Dicotyledons (which is greatly facilitated by the excellent illustrations in Hutchinson's work (5)) leaves the impression that pentamery is an advanced, specialised and singular case. The perfectly pentamerous flower appears to be far more common in the botanical text-books than it is in nature. To apply principles discovered by a consideration of this special and unusual case to all the flowers of the Dicotyledons, is an inductive argument from the particular to the general which will be found difficult to sustain. Even if pentamery be the general rule in the perianth, what grounds are there for presuming that the androecium must follow the same line of evolution? Unless it can be shown that the relation between a petal and an opposed or alternate stamen, is functional and not simply positional, the conception of a "petal stamen", or a "sepal stamen", has little to commend it.

The free, many-stamened androecium of the Tiliaceae may be expected to evolve along one, two, or all of the three lines: adnation, connation, and reduction. As the stamens are united in all the other families of the group, it may be said that it *always* evolves along the line of *connation*. In the Bombacaceae and the Malvaceae it is not reduced in numbers, and the stamens are seldom reduced to stami-

nōdes; but the androecium becomes *adnate* to the petals at their bases. In the Buettneriaceae and the Sterculiaceae there is no adnation, but the stamens are *reduced* in numbers or become staminodial. These are, therefore, two distinct lines of evolution of the androecium in the Malvales. The unilocular anther also marks out the Bombacaceae and Malvaceae as being on a line of evolution different from that of the Buettneriaceae and Sterculiaceae.

In general, the androecium of the Sterculiaceae differs from that of the Buettneriaceae in its lack of staminodes, and in its less perfect connation accompanied by an often irregular arrangement of the more numerous stamens. Thus, it seems to be more primitive than that of the Buettneriaceae. Similarly, the androecium of the Bombacaceae is less connate than that of the Malvaceae, and it often shows distinct traces of the pentamerous polyandry which characterises the Tiliaceae; further, the anthers are sometimes bilocular (as in *Bombax malabaricum*). The Malvaceae are thus more advanced than the Bombacaceae.

In the Sterculiaceae and Malvaceae the unusual states of apocarp and schizocarp respectively are found. These can only be regarded as advanced forms, but as the one is due to the growing apart of contiguous carpels, and the other to the fission of a gynoeceum that is really united, they have advanced along differing lines.

It is hard to imagine that the syncarpous Buettneriaceae were derived from the apocarpous Sterculiaceae, and these derived in turn from the syncarpous Tiliaceae; nor can one conceive of the Sterculiaceae, with their more primitive androecium, being derived from the Buettneriaceae; therefore, the two groups Sterculiaceae and Buettneriaceae must have evolved from the Tiliaceae along mutually independent lines. This conclusion is borne out by the apetalous and unisexual flowers of the Sterculiaceae; and the genus *Christiana* indicates the line of evolution from the Tiliaceae to the Sterculiaceae. The schizocarpous gynoeceum of the Malvaceae, however, must have been derived from a syncarpous form, so that the Malvaceae are probably more advanced than, but in the same linear sequence as, the Bombacaceae.

In general then, the phylogeny of the families of the Malvales in relation to one another, is as follows: the Scytopetalaceae are relics of those plants which once linked the Tiliaceae with more primitive groups, such as the Dilleniaceae and the Magnoliaceae. The Tiliaceae are the oldest, most primitive, and largest of the larger families. The Sterculiaceae are a small and rather specialised group, derived

directly from the Tiliaceae. The Buettneriaceae group of tribes were likewise derived from the Tiliaceae, and show considerable evolution from polyandrous multi-staminate, staminodial forms (as *Cistanthera*) to few-staminate, monadelphous, astaminodial forms (as *Waltheria*); this evolution is accompanied by a gradual change from an arborescent to a herbaceous habit. The Bombacaceae are derived from the Tiliaceae along a different line, and are characterised by the development of unilocular anthers, and a connate and adnate androecium. They lead on to the Malvaceae, which are a highly advanced herbaceous family with a modified schizocarpous gynoecium. In so far as the Euphorbiaceae are derived from the Malvales, their point of origin is probably in the region of that of the Bombacaceae (compare *Kydia*).

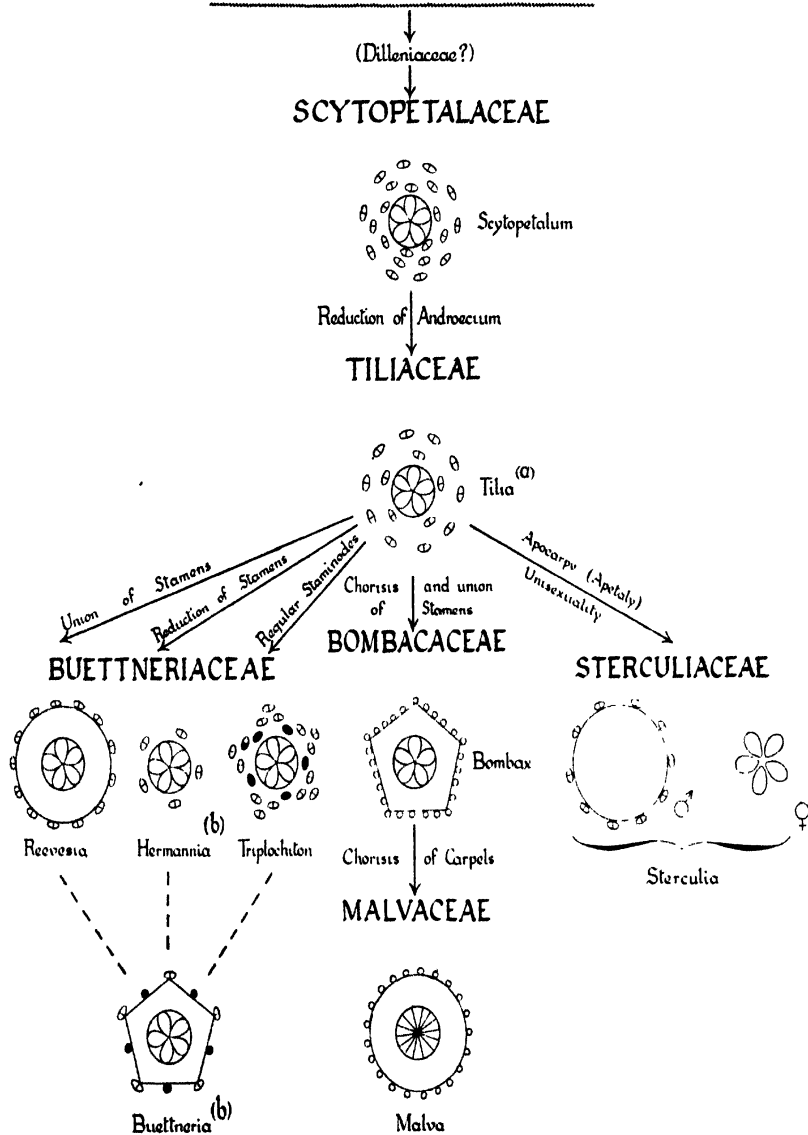
GENERAL CONCLUSIONS AND SUGGESTIONS

Examination of herbarium specimens, together with a consideration of the systematic anatomy and wood structure, shows that the existing classification within the group Malvales is unsatisfactory. A review of former classifications shows that diversity of opinion exists among the authorities.

After consideration of many atypical genera, it is found that all the Malvales may be referred to definable families, and that a practicable key may be drawn up for these. On this basis, the probable phylogeny of the Malvales has been worked out. The two families Scytopetalaceae and Tiliaceae appear to be the oldest and most primitive of the Malvales. The three families Sterculiaceae, Buettneriaceae, and Bombacaceae, appear to have evolved from the Tiliaceae upon mutually independent lines. The Malvaceae proper are a small advanced group derived from the Bombacaceae.

In particular it is suggested that the family Gonystylaceae, with the single genus *Gonystylus*, should be merged in the family Scytopetalaceae. That the Elaeocarpaceae should become a tribe of the family Tiliaceae. That the family Chlaenaceae should be entirely excluded from the Malvales. That the tribe Sterculieae should stand apart as a distinct family, Sterculiaceae; and that the name Buettneriaceae should be applied to the remainder of the family at present called "Sterculiaceae". That the tribe Hibisceae, together with certain atypical genera of the Malvaceae, as formerly delimited by Bentham and Hooker, should be transferred from that family to the Bombacaceae. That the genus *Kydia* should form a distinct tribe, Kydieae, in the family Bombacaceae. The tribe Fremontieae, with

THE PHYLOGENY OF THE MALVALES



the two monotypic genera *Fremontia* and *Cheirostemon*, should be placed in the family Bombacaceae.

Other conclusions and proposed alterations are: *Prockia*, *Hasseltia*, and *Plagiopteron*, remain in the Tiliaceae, but *Ropalocarpus* has been excluded. The division of *Grewia* into two genera, *Grewia* L. and *Microcos* Burret, as proposed by Burret, appears to be sound, but there are insufficient grounds for his isolation of a third genus, *Vincentia* Boj. The family Sterculiaceae falls naturally into two tribes: the Tarrietieae include the three genera *Tarrietia*, *Heritiera*, and *Argyrodendron*; the other genera are referred to the Sterculineae. The Australian species of *Tarrietia* are placed in the distinct genus *Argyrodendron* F.v.M.; the name *Argyrodendron trifoliatum* F.v.M. is revived for the species also called *Tarrietia argyrodendron* Benth.; the name ***Argyrodendron actinophyllum*** (Moore) Edlin nov. comb., is proposed for the species hitherto called *Tarrietia actinophylla* Moore.

Cistanthra is placed with *Triplochiton* and *Mansonia* in the tribe Mansonieae of the Buettneriaceae. *Humbertiella* Hochr. is transferred from the Malvaceae to the tribe Dombeyeae of the Buettneriaceae. The genera *Howittia*, and *Hampea*, are referred to the tribe Hibisceae (Bombacaceae). *Bernoullia* Oliv. is placed in the tribe Adansonieae (Bombacaceae). *Nettoa* Baill., and *Hua* Pierre ex de Wild. are excluded from the order Malvales.

NOTE ON THE CYTOLOGY OF THE MALVALES

Dr Hugh Davie, in the *Journal of Genetics* (28, 33 (1933)), gives an account of the chromosome constitution of certain genera of the Malvales. He finds that many species of the tribes Malveae and Malopeae have the basic chromosome number 7, while *Abutilon*, certain other Malvaceae, the Hibisceae, and *Theobroma*, have higher basic numbers, ranging from 8 to 13.

His suggestion that the Malvaceae, with higher chromosome numbers, are more primitive phylogenetically than the Malopeae and Malveae, which have only 7, is directly opposed to the conclusion reached in this paper, that these two tribes are the most advanced of all the Malvales.

ACKNOWLEDGMENTS

The writer wishes to express his thanks to the Director and Staff of the Imperial Forestry Institute, under whose auspices this in-

vestigation was undertaken; and in particular to Dr J. Burt Davy, and Mr A. C. Hoyle, of the Section of Systematic Botany, under whose guidance it has been carried out; to Dr L. Chalk, and Miss Margaret M. Chattaway, for information concerning wood structure; to Mr H. Baker, Librarian of the Oxford University Department of Botany, and Prof. M. Drummond of Manchester University, for their courtesy in allowing the use of works of reference; and to the Director and Mr A. D. Cotton, Keeper of the Herbarium, Royal Botanic Gardens, Kew, for facilities in the examination of Kew material.

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STRUCTURE AND DEVELOPMENT OF THE SYNERGIDS IN *AMMANIA BACCIFERA* LINN.

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(With Plate III, containing Figs. 1-10)

THE following communication relating to the structure and development of the synergids in the embryo-sac of *Ammania baccifera* Linn., a marsh annual herb belonging to the family Lythraceae, is being written on account of the exceptional behaviour of these parts of the embryo-sac in this plant. This fact was recorded by us some time ago in a brief note elsewhere(4). Details and figures are given in the present paper.

The ovules of *A. baccifera* are of the normal anatropous form with two integuments and a moderate amount of nucellus. They show many primary archesporial cells, one of which usually develops to form the primary parietal cell and the megaspore mother cell. The latter gives rise to four or three megaspores. Out of these, the chalazal megaspore is always the functional one and develops into the 8-nucleate embryo-sac in the normal manner, forming three antipodals, an egg apparatus of an egg cell and two synergids and two polar nuclei. A tapetum is differentiated even at the megaspore mother cell stage and persists up to the complete formation of the embryo-sac. The embryo-sac is at first narrow at both the ends but later on broadens at the micropylar end. The antipodals are at first arranged in a row, but in the mature embryo-sac they form a triangle. The polar nuclei take their place near the egg apparatus. The egg cell has the normal structure and shows a large vacuole towards the micropylar end and the nucleus at the lower end.

The synergids, on the other hand, differ markedly from those of other flowering plants investigated so far. At an early stage in the development of the embryo-sac, soon after the 8-free-nucleate stage and much before the egg cell is ready for fertilisation, the two synergids are quite free from each other. They lie side by side in their usual position and in frontal view completely cover the egg cell, except at its lower end (Fig. 1). The size of the egg cell and the synergids is about the same, both being about 6μ in length. Laterally the syner-

gids touch each other on one side, but are separate on the opposite side. A peculiarity of the synergids visible even at this stage and one that persists throughout their later life is the absence of vacuoles. As a rule, there is no trace of them. Only in one instance, a small vacuole in one of the synergids has been seen (Fig. 3). Correlated with the above fact, the nuclei of the synergids are not situated close to their micropylar ends. They are found about the middle (Fig. 2) or even somewhat near the chalazal end. The whole of the plasma of the synergids is uniformly very dense and stains deeply.

The free condition of the synergids lasts for a very short time. After examining hundreds of ovules, we have seen it only in a few instances. Very soon they begin to grow in size and to press upon each other laterally. Ultimately the line separating them vanishes (Fig. 2) and they fuse with each other (Figs. 2 and 3). The fused structure, which may be now called a "syn-synergid", enlarges very much in size, keeping pace with the growth of the egg cell and the embryo. At certain stages, it is even larger than the egg cell (Fig. 3). The free synergids (Fig. 1) are only about 6μ long. By the time the fertilisation takes place (Fig. 4), the syn-synergid is about 25μ in length and diameter. Fully formed synergids measure from 50 to 60μ in length and breadth (Figs. 6 and 7).

The syn-synergid covers the egg and later on the proembryo and the embryo on one side (front side) completely (Fig. 6) and on the opposite side partially. Around the suspensor of an embryo, the syn-synergid is equally broad all round, but lower down about the base of the embryonal mass it is much thicker on one side than on the other. This can be seen from Figs. 8 and 9, which represent cross-sections of a syn-synergid, one at the level of the suspensor (Fig. 8) and the other at the level of the embryonal mass (Fig. 9). A complete diagram of the syn-synergid is given in Fig. 5. This is a reconstruction from two adjacent sections and shows the synergids at the three-celled stage of the proembryo in the sagittal plane. The whole thing looks like a collar with one side broader than the other, surrounding the base of the embryo, with a narrow opening at the top into which the micropylar end of the embryo fits and a broad opening on the opposite side through which the embryonal cell (and, later on, the embryonal mass) projects.

The syn-synergid is binucleate when it is just formed, one nucleus coming from each synergid. These nuclei are, at first, in their original position, about the middle of each synergid, but now they move downwards towards their base (Fig. 3). They show a gradual increase

in size, and ultimately begin to divide and multiply in number as the syn-synergid rapidly enlarges. The syn-synergid thus becomes a multinucleate coenocyte, the number of nuclei reaching about 20. From 15 to 17 nuclei in a syn-synergid just as the periblem and plerome are forming in the embryo (Fig. 6) are quite common. The divisions that give rise to such a large number of nuclei from the original two, take place amitotically by a process of budding (Figs. 5, 6 and 9). During this process, the nuclear cavity of the mother nucleus becomes perfectly hyaline, probably due to the flow of the whole of the chromatic material into the nucleolus. This nucleolus buds off one or more round daughter nucleoli resulting in the presence of two or more daughter nucleoli within the cavity of the mother nucleus. Constrictions in the wall of the mother nucleolus now appear around the individual daughter nucleoli. These gradually increase in size and lead ultimately to the separation of the daughter nuclei from the mother nucleus. These daughter nuclei now move apart and repeat the process. As the syn-synergid reaches its maximum size, multiplication in the number of the nuclei stops. These now begin to grow in size, reaching a diameter of 8μ and develop a faint chromatin reticulum (Fig. 7).

The nuclei in the syn-synergid are distributed more abundantly on one side than on the other (Figs. 5, 6, 8 and 9). This is the frontal side on which the synergids previously in the free condition were situated and on which side the syn-synergid is thicker (Fig. 9). This unequal distribution of the nuclei in the syn-synergid is quite natural, as the two parent nuclei were on this side and all the daughter nuclei have radiated out from them.

Finally, the syn-synergid degenerates when the embryo has reached the stage represented in Fig. 10. Dermatogen, periblem and plerome have become fully established at this stage in the embryonal mass. The hypophysis cell has divided into two and one of its daughter cells has already divided longitudinally.

The above behaviour of the synergids in the embryo-sac of *Ammania baccifera* has been found to be quite constant, and no exception has been observed so far. The process starts, as said previously, at a very early stage, much before the beginning of the process of fertilisation and not after the completion of this process, as was stated by us previously⁽¹⁾. This can be seen from Fig. 4, which represents a stage in the process of fertilisation. The section is from one side of the egg apparatus and the egg cell and the synergids have not been cut in the median plane. The apex of the stout pollen tube

is still intact and it has not as yet discharged its contents. The synergids have already fused, measure about 25μ in length and diameter, and on counting 9 nuclei have been seen in this particular case.

In preparations stained with Haidenhain's iron alum haematoxylin, during early stages, the syn-synergid and the embryo take nearly the same amount of stain. The rest of the embryo-sac stains very lightly. During later stages, as the time of their degeneration draws near, the staining capacity of the synergids decreases. A similar reaction is given by iodine solution. This stains the synergids and the embryo yellowish brown, indicating only the presence of dense plasma. Starch grains are totally absent from these two parts, though they are present in other parts of the ovule, particularly in the neighbourhood of the vascular elements in the funicle.

COMPARISON AND DISCUSSION

In general, the synergids in the embryo-sac of angiosperms, with regard to the method of their formation, structure and position, show very little variation. Their position at the apex of the embryo-sac, their vacuoles situated towards their chalazal ends and the position of their nuclei in the micropylar region, are peculiarities which have been found to reappear with very few exceptions in the numerous flowering plants which have been investigated by now. In most instances further, the synergids have been found to degenerate as soon as the process of fertilisation is complete. For these reasons any variation in the structure of the synergids is worth recording.

The chief features of the synergidae of *Ammania baccifera* are the absence of vacuoles, their great increase in size, their fusion with each other to form a single structure, the multiplication of their nuclei by a process of amitosis and their persistence up to a fairly late stage of embryo formation. It may be immediately stated, that so far as the writers are aware, these features all together have not been recorded in any other flowering plant. In the family Lythraceae, the embryo-sac of *Lythrum Salicaria* has been studied in detail by Tischler (14) and he has also made some comparative observations on species of *Cuphea*. The embryo-sac of some more species of *Cuphea* has been studied by Jönsson (3) and Guignard (1). We have ourselves by now examined the embryo-sac of *Lawsonia alba*, *Woodfordia floribunda*, and *Lagerstroemia Flos-reginae*. In all these plants normal synergids have been found to occur. The morphology and cytology of several

other families of the Myrtales is well known, and although several other anomalies have been observed in their embryo-sacs—e.g. the embryo-sac in the Onagraceae is only 4-nucleate (2, 6, 8), in the Penaeaceae it is 16-nucleate (12), etc.—but in no case synergids of the type, such as have been found in *Ammania baccifera*, have been reported. A comparison in some respects, however, can be made with certain unrelated plants.

The absence of vacuoles in the synergids is a very rare feature, but synergids which completely lack the vacuoles are present in *Limnanthes Douglasii*, according to Stenar (11). The lower part of the synergids in this plant is spherical and swollen, and is filled completely with dense plasma. Also in this plant the nuclei show hypertrophic development. In *Lycopsis arvensis*, the conditions, according to Svenson (13), are somewhat similar. The synergids are more strongly developed than the egg cell and possess very small and not sharply defined vacuoles of varying position, and their nuclei are early hypertrophied.

The enlargement and persistence of the synergids are already known in *Trapella*. According to Oliver (5), the synergids in this plant after fertilisation increase much in size and in the mature seed form a conspicuous tubercle-like body. Schürhoff (9) has similarly observed the persistence, strong growth and intense stainability of the synergids and a great increase in the size of their nuclei, often becoming bigger than the complete synergid in the beginning, in *Allium odorum*.

The fusion of the two synergids at a very early stage and a large multiplication in the number of their nuclei, however, has been observed in no other plant, and in these respects the synergids of *Ammania baccifera* appear to be unique.

The function of this peculiar development of synergids in *Ammania baccifera* seems to be obscure, and it is rather difficult to put forward any definite view. In the various examples in which the synergids have been found to persist, enlarge in size, have deeply staining plasma, no vacuoles and nuclei showing hypertrophy, it has been generally believed that they help in the nutrition of the egg, Schnarf (7). In *Allium odorum*, for instance, the endosperm develops very late, after the embryo has already undergone a number of cell divisions and Schürhoff (9) assumes that these synergids take over the function of endosperm. In *Ammania baccifera* the endosperm is very scantily developed, and during early stages of embryogeny, while the rest of the embryo-sac is very poor in plasma, the synergids

stain as deeply as the egg cell or the embryo, both with haematoxylin and iodine solution. It is, therefore, quite probable that the synergids in this plant also may be playing some role in the nutrition of the egg.

As regards the occurrence of amitosis, it is usually associated with tissues having a nutritive function or with pathological and degenerating tissues. It has, therefore, been put forward by some workers that amitosis aids in the process of metabolism by increasing the nuclear surface and by others that it is primarily a degenerative phenomenon, Sharp(10). The synergids of *Ammania baccifera*, as stated above, may be for some time performing a nutritive function. Ultimately they degenerate. The amitotic multiplication of nuclei in them may be, therefore, correlated with both of the above explanations and its occurrence may be regarded as quite expected.

SUMMARY

The embryo-sac of *Ammania baccifera* develops from the chalazal megaspore in the normal manner. It is of the 8-nucleate type. All its parts have the usual structure except the synergids. Their free condition, which is normal in other plants, lasts only for a short time. Very soon they fuse with each other laterally and form a sort of collar around the egg and later on around the base of the growing embryo. They show great increase in size from about 6 to 60 μ . They are peculiar in the absence of vacuoles and presence of a uniform dense plasma, in the multiplication of their nuclei by amitotic divisions and in persisting up to a fairly late stage of embryo development. Such a behaviour of synergids has not so far been recorded in any other flowering plant.

Note on the figures. All the figures are camera lucida drawings, but Figs. 5 and 6 are reconstructions from a number of adjacent sections to show the whole form of the syn-synergid and the total number of nuclei. The embryo figures are not shaded. Magnification of all the figures is about 1150 times.

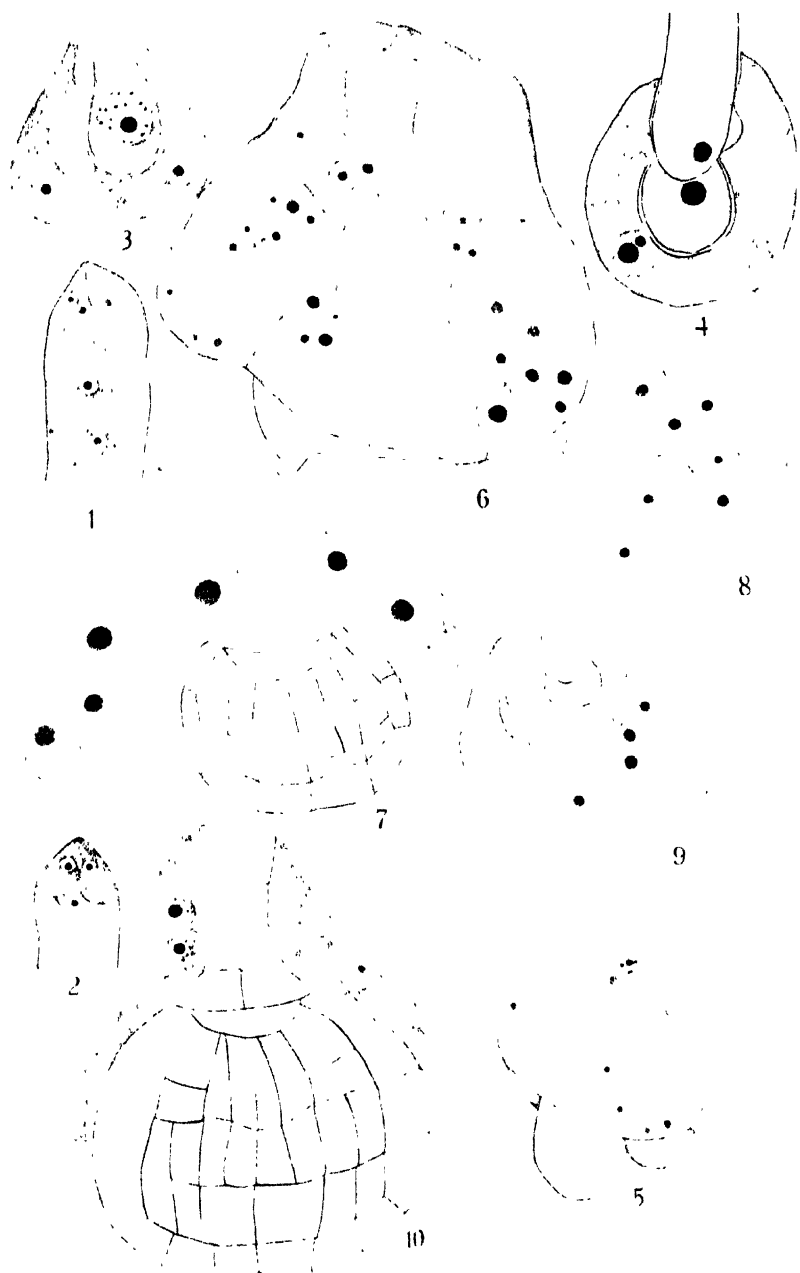
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EXPLANATION OF PLATE III

- Fig 1. *Ammania baccifera*. Micropylar part of the embryo-sac, showing the egg apparatus and two polar nuclei. The synergids as yet are quite free from each other.
- Fig 2. A slightly later stage than that shown in Fig 1; the line separating the two synergids has dissolved and they have fused.
- Fig. 3. The egg apparatus at a stage later than the one represented in Fig. 2, cut from one side, not medianly. The synergids are now much larger and fused with each other. One of them shows the exceptional presence of a vacuole. There are two nuclei towards the base.
- Fig. 4. A stage in the process of fertilisation as seen from one side. The synergids at this stage show about 9 nuclei.
- Fig. 5. The syn-synergid at the 3-celled stage of the proembryo in sagittal view.
- Figs. 6 and 7. Later stages in the development of the syn-synergid.
- Figs. 8 and 9. Cross-sections of one syn-synergid. Fig. 8. At the level of the suspensor. Fig. 9. About the base of the embryonal mass. Fig 9 shows that at this level the syn-synergid is much thicker on one side than on the other. Both the figures show that nuclei are more abundant on one side (thicker side) than on the other.
- Fig. 10. Syn-synergid at the time of degeneration.



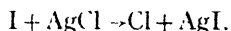
THE ESTIMATION OF SMALL AMOUNTS OF CHLORIDE IN PLANT TISSUES

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IN order to pursue quantitatively a study of the effect of chlorides on the diastase complex (James and Cattle, 1933), it was necessary to find a reliable method for the estimation of small amounts of chloride in plant tissues and digests. The standard methods for chlorine determination were found to be unsuitable as they took too much time and material and gave too great an error. One of the principal sources of error in the older methods was the loss of chloride during ignition. Extracts of leaf material were, therefore, made by acid and alkaline "wet-ashing" methods, the choice between acid and alkaline depending upon the particular method of chloride estimation to be used on the extract.

Titrimetric methods are rapid in execution, and an attempt was made to use the extreme sensitivity of the iodometric method. The chloride was precipitated as silver chloride by the addition of excess silver nitrate, and after filtration to remove the silver chloride, the residual silver nitrate back-titrated with potassium iodide using starch as indicator. The filtration was necessary, since there is a great difference between the solubilities of silver iodide and silver chloride, and without filtration the blue colour does not appear until all the silver chloride has reacted as follows.



The leaf material had been dried at 30° C. and powdered, and a solution containing chloride was prepared from it by alkaline "wet-ashing", since the iodometric titration cannot be carried out after a nitric acid preparation. This necessitated prolonged heating with a concentrated solution of potassium hydroxide with repeated addition of perhydrol (hydrogen peroxide, 100 vol.) to complete the breakdown of the more resistant substances. The process was carried out in a round flask, of a large size relative to the volume of the mixture owing to the vigorous frothing that occurred at high temperatures. A clear liquid was not obtained and the solid residue was, therefore, filtered off, and the extract made up to a known volume before

titrating. In this way 50 c.c. of extract were obtained from 0.1 gm. plant material—sufficient for several titrations.

The preliminary work for the iodometric titration was carried out with potato leaves, and a good end-point was obtained and reasonable results. When, however, the method was used with extracts of bean leaves it was quite impossible to obtain an end-point as the colour faded very rapidly. The iodometric titration was then abandoned as a general method for the determination of chlorides in plant tissues, and since there was no objection to an acid extraction for a modified Vohlhardt's titration (described in detail below) digestion with concentrated nitric acid rather than potassium hydroxide was used. The method eventually adopted was modified from a clinical method (Peters and van Slyke, 1932) brought to our notice by the Oxford Biochemistry Department.

PROCEDURE

Solutions

(1) *Silver nitrate in nitric acid.* The solution is made up accurately 0.05 *N*. The silver nitrate should be dissolved in the minimum amount of water, and the volume then made up with concentrated nitric acid.

(2) *Thiocyanate solution.* 1.6 gm. of sodium thiocyanate and 1.5 gm. of ammonium thiocyanate are dissolved in about 1 litre of water, and the solution standardised against the silver nitrate solution.

Preparation of extract by acid "wet-ashing"

(1) 0.05 gm. of dry leaf material is ground to a paste with water in a very small mortar, and washed into a boiling tube, using the minimum amount of water.

(2) Five drops of capryl alcohol are added to stop frothing and the mixture boiled (with a suction tube inserted into the mouth of the boiling tube to prevent the escape of fumes) to wet the material thoroughly and to concentrate it.

(3) 2 c.c. of 0.05 *N* silver nitrate are added and then 2 c.c. of nitric acid, and the mixture boiled. The silver nitrate and nitric acid are thus combined in a single solution which will keep indefinitely. It is important to add the silver nitrate solution before the pure nitric acid to prevent loss of chloride by volatilisation.

(4) Ten drops of perhydrol and five drops of capryl alcohol are added and the liquid boiled. The addition of perhydrol completes the

breakdown of the plant material, at the same time decolorising the suspension (necessary for the subsequent titration) and any excess can be boiled off. Perhydrol is preferred to potassium permanganate, as excess of the reagent is thus removed without the addition of any other substance to the sample.

(5) The sides of the tube are washed down with distilled water and the process repeated from (4).

(6) The solution is concentrated to about 7 c.c. and then titrated.

Titration

5 c.c. of a 6 per cent. solution of ferric alum are added as indicator, and the excess silver nitrate titrated with thiocyanate until the first appearance of a pink colour that persists for 15 sec. Blank titrations with 2 c.c. 0.05 *N* silver nitrate are performed, and the chloride content of the experimental solutions calculated from the difference between the two titrations. The tube must be shaken vigorously during the titration and a temporary excess of the ferric salt must not be tolerated. Such an excess was found to give a percentage error of from 3 to 4 per cent.

The chloride equivalent to 1 c.c. thiocyanate is:

$$\text{c.c. thiocyanate} \equiv 1 \text{ cc. } 0.05N \text{ silver nitrate solution} \cdot \frac{1.773}{\dots}$$

EXPERIMENTAL RESULTS

Preliminary experiments were performed using both acid and alkaline digestion of the material (alkaline extracts were acidified with nitric acid before titrating), and with pure and accurate volumetric solutions of chloride, with leaf material and with a mixture of chloride solutions and leaf material. The results are summarised as follows:

TABLE I

Values obtained in a standardisation experiment, using accurate potassium chloride solutions

Mg. chloride in solution	2.95	2.22	1.48	1.11	0.74	0.55	0.44
Mg. found by titration	2.92	2.21	1.47	1.11	0.74	0.55	0.45

The titration method was found to give an accurate measure of the chloride content of solutions (Table I), and to be unaffected by

TABLE II

Tests of the preparation method

Material used	Method of preparation	Chloride found mg.
5 c.c. sodium chloride solution	None	1.45
	Acid "wet-ashing"	1.45
	Alkaline "	1.47
0.05 gm. leaf	Acid "	0.61
	Alkaline "	0.60
0.05 gm. leaf + 5 c.c. solution	Acid "	2.06
	Alkaline "	2.06

the method of preparation of the material and by the presence of actual leaf material (Table II). The individual readings (not tabulated) were more regular with the acid preparation than with the alkaline method, and for this and other reasons acid "wet-ashing" was adopted for the preparation of an extract. The advantages of this method are considered to be as follows:

(1) Acid "wet-ashing" is much quicker than alkaline "wet-ashing".

(2) There is no need for the removal of the silver chloride by filtration, as the nitric acid and capryl alcohol coagulate it very thoroughly during digestion.

(3) In an acid preparation the whole procedure is carried out in one tube. In an alkaline preparation, repeated transference of material and the large size of the boiling flask allow of greater possibilities of error.

(4) If the silver nitrate and the nitric acid be combined in one solution serving for digestion and chloride precipitation, there is the additional advantage that the solution keeps indefinitely.

This method of chloride estimation was worked out in the course of an investigation financed by a grant to Dr W. O. James from the Department of Scientific and Industrial Research.

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NOTE ON THE ANALYSIS OF CHLORIDE
IN *CLADOPHORA*By E. W. JONES
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During some investigations on ionic uptake which were being carried out at the Botany School, Cambridge, it was necessary to analyse the fresh-water alga *Cladophora glomerata* for chloride. Miss Cattle kindly gave me the details of the alkaline wet-ashing technique which she was then using for potato leaves, and I was able to use the essential part of this method—the oxidation of the organic matter by perhydrol—successfully.

The amount of chloride present in *Cladophora* is very small—for the expressed sap it is about 0.1 mg. per c.c.—and the total amount of chloride available for estimation was about 0.05–0.1 mg. Since these amounts were rather small for titration, the actual estimation was done nephelometrically, according to the procedure of Lamb, Carleton and Meldrum (1929). By this method quantities of chloride of this order can be estimated with an extreme variation of 5 per cent.

At first the method of ashing used was exactly as described by Miss Cattle, but later the following procedure was adopted. The material was washed into a beaker or evaporating dish, concentrated potassium hydroxide solution added and the mixture boiled well. When cool, 0.5 c.c. of perhydrol was added, and allowed to stand for 24 hours or more. If necessary a further lot of perhydrol may be added, until a clear, pale green solution is obtained. This was then warmed to expel excess perhydrol, carefully neutralised with concentrated nitric acid, and the solution filtered off into a measuring flask and made up to 25 c.c. Aliquot parts of this solution were then used for the estimation. Whilst this procedure takes longer than that originally described, it is less trouble and several estimations can be done together.

The nephelometric estimation is carried out in a 50 per cent. alcoholic solution, and if too much potassium hydroxide has been used originally, there is a danger that a small amount of the potassium nitrate which is present may be precipitated by the alcohol and produce a cloudiness. This can be guarded against by adding alcohol after the neutralisation, and washing and making up to the mark with 50 per cent. alcohol.

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REVIEWS

Scolt Head Island. Edited by J. A. STEERS. Pp. 234, 34 plates, 17 text-figures and 1 map. W. Heffer and Sons, Ltd. 15s. net

The interest of the north Norfolk coast to students of maritime and dynamic ecology is great, and it is accordingly a matter of considerable satisfaction that two of the most striking areas on that coast, Blakeney Point and Scolt Head Island, are now preserved in a natural condition by the National Trust. It is equally a matter of satisfaction that a detailed account of the physiography, fauna and flora of Scolt Head Island is now available in the present volume.

Almost the whole of this work is of interest to the botanist. There is a noteworthy account of the history, evolution and physiography of the island by J. A. Steers, which is also valuable because it gives an excellent idea of the methods to be employed in tackling physiographical problems, especially, perhaps, when it is read in conjunction with Prof. Debenham's article on photographic methods used in the geographical survey.

The island is assumed to have originated in an offshore sand bar, which gradually accumulated a pebble beach. The latter subsequently became capped by sand-dunes and developed salt-marshes on its inner side. Extension is now mainly towards the west and has been shown to be due to wave action rather than to tidal currents. Near the channel separating the island from the mainland, three peat beds of different ages are exposed. By the method of pollen analysis, these show that the shoreline has probably been subsiding (with minor periods of elevation), since Boreal times. One of the features of the uppermost peat bed is the great relative abundance of *Pinus* pollen, and Dr and Mrs Godwin suggest that this is either a recent peat affected by local pine plantations or else it must be correlated with a pine maximum traceable in the fenlands at some period between the Bronze Age and the Romano-British Era. In support of the latter conclusion, they record a similar peat, rich in pine pollen, from the coast at Ingoldmells, which on cultural evidence is probably of Bronze Age.

The vegetation of the island is that of the shingle beach, drift line, sand-dunes and salt-marsh. It is described in detail by V. J. Chapman and shows the general features common to other similar areas. Full details make the account valuable for comparative purposes. The salt-marshes show variations of considerable interest, while the general succession of dominants is not unusual, namely, *Zostera* → *Salicornia* → *Aster* → *Armeria* → Sea Meadow (*Glyceria-Obione*) → *Juncus maritimus* → Fresh-water marsh. Interesting variations are described when mud is partly or largely replaced by sand. There is further an unusual community dominated by *Plantago maritima*. In addition to the details of these variations, Mr Chapman gives a detailed and valuable analysis of a problem which, previously, has not been exhaustively considered in this country. He describes the vertical ranges of the salt-marsh species and communities and their relation to the tidal levels. His analysis enables him to state how often each type of vegetation is submerged, how often it is submerged during the daytime and when it is exposed to the air for the longest periods. The detailed tables and graphs summarising his conclusions are of the greatest interest to maritime ecologists. They enable him to point out that the high-level marshes differ from those at lower levels, in having long periods of exposure in summer and most frequent submergences during the beginning of the growth season and the time of fruit dispersal. Further, none of the higher plants are found below mean sea-level. Even *Zostera nana* is commonly sub-

merged for less than half the time. We may congratulate Mr Chapman on a very useful contribution to salt-marsh ecology. At the same time, it would have been interesting to have had the author's views on some of the other facts which emerge from his tables, notably, for example, the fact that the general salt-marsh (*Armerietum*) and sea meadow appear to possess very similar vertical ranges. Further, Mr Chapman's methods of expressing the habitat characteristics draws attention to the fact that the higher salt-marsh communities may commonly be free from submergence for periods as long as twenty-five days. It seems clear that in such cases the water relations of the soil are less directly related to tides. Possibly surface drainage and "banking" effects of tides may become all important. These factors certainly affect the vertical ranges of the species. On most higher salt-marshes, there is a very evident gradation of species from the centre of each sector towards the drainage channels. In some cases, almost all the salt-marsh communities from *Salicornia* to meadow may be seen at the same level, though the soil water is normally at different levels depending on the distance from the drainage channels. These effects apparently account for much of the large vertical range of many salt-marsh species, and may ultimately prove to bear on the development of fresh-water marsh which, it is suggested, may succeed the maritime communities on this island.

The botanical part of this book is concluded by accounts of the Bryophyta by C. V. B. Marquand and of the lichens by C. J. Dickinson. These are arranged so that they form a profitable extension of the ecological section, and they illustrate clearly the importance of these smaller plants in the general scheme of succession. The whole volume, therefore, is a very valuable addition to our ecological literature, especially for those who are interested in the maritime or dynamic aspects of the subject.

W. H. PEARSALL.

Une Relique de la Sapinière Méditerranéenne, le Mont Babor Monographie de l'Abies numidica Lann. Étude de Sylviculture, de Dendrologie et d'Entomologie forestière. A. BARBEY. Pp. 82+xx, 33 plates. Belgium: Duculot, Gembloux. 1934.

Pitched high on the north slopes of Mount Babor in the Kabylie Range in Algeria, *Abies numidica* makes its last stand against the attacks of man and his domestic animals. Its survival here is due to its inaccessibility. deep snow in winter and steep slopes and broken country in summer discourage alike the tourist and the indigenous inhabitants in search of pastures new.

The forest of *A. numidica* occupies an area of 6-7 by 1½ kilometres at an elevation from about 1700 m. to the summit of Babor at 2004 m. It contains some 2000-3000 stems of 20 cm. diameter and over at breast height, so that it is a more substantial relict than the cedar forest of Lebanon (under 400 trees and none less than 50 years old) and of *A. nebrodensis* in Sicily now represented by one tree. More drought resistant and light demanding than most firs, *numidica* is not a social species (groups of more than five to eight rarely occur); it is, in fact, a constituent of a mixed forest whose chief tree species besides the fir are *Cedrus atlantica* and *Quercus mirbeckii*. Other species less numerous are *Acer obtusatum*, *A. campestre* and *A. monspessulanum*, *Populus tremula*, *Sorbus torminalis*, *S. aria* and *Taxus baccata*. Shrubs include *Ilex aquifolium*, *Gemsta tricuspidata*, *Crataegus oxyacantha*, *Rhamnus catharticus*, and *Juniperus oxycedrus*. The conditions at this altitude are less than optimal: the branches of the fir are broken and twisted by snow, wind and frost and from a forester's standpoint the state of the forest is deplorable. Regeneration is impossible on denuded sterile soil, and the humus of *Q. mirbeckii* seems necessary for the germination and establishment of the fir seedling.

This mixed forest contrasts with the relative purity of the cedar forest (much destroyed by fire and by indiscriminate and wasteful exploitation) and of *Quercus ilex* forest passed in descending to the limits of cultivation. The whole is set in a matrix of much eroded country studded with bush xerophytes.

About one-third of the book is devoted to morphological and biological notes on insects found on *Abies numidica*, and comparison is made with the insect fauna of *A. pinsapo* and *A. alba (pectinata)*. The author's study exonerates harmful insects from blame for the present condition of the forest and eliminates them as a cause of its possible extermination. In his mind there is no doubt about the factors likely to destroy the forest.

It has been said that goats are responsible for the Sahara, and if the claim is an exaggeration, there is a kernel of truth in it. Quite recently attention has been called to the rapid extension of the desert southwards due to overgrazing. The bill of indictment against grazing animals is formidable: all Mediterranean countries bear witness to it. Man, the agriculturist, has ever regarded the forest as a mistress to be enjoyed and the wreckage abandoned at his pleasure regardless of the repercussions on himself, his neighbour and his successors: man, the industrialist, treasures the forest as a useful helpmate. The relative importance of agriculture and the heavy industries in Mediterranean countries, both in the past and now, accounts for the small area under forest (2 per cent.). How great has been the effect of forest destruction on the physiognomy of Mediterranean vegetation cannot now be known, but it is permissible to imagine a climax with more trees in it if not more tree species than now exist. How great has been the repercussion of forest destruction on agriculture may best be appreciated by reference to the findings of the Committee of Reconstruction of Palestine and its recommendation to build again the old waste places as a check to further erosion and a preliminary step to the restoration of soil fertility.

As to the effect of man on the number of species of *Abies* in Mediterranean lands, comparison may be made with *Abies alba* of Central Europe. It is a single species, more or less continuous throughout its range, but, as foresters know well, there are many ecotypes. In the Mediterranean there exist in isolated places and covering usually relatively small areas, *A. pinsapo* and its African variety *marocana*, *A. numidica*, *A. nebrodensis*, *A. cephalonica*, *A. cilicica*, *A. nordmanniana* and its varieties *equi-trojani* and *bornmulleriana*. These have a common ancestor in the Tertiary *A. intermedia*, and one may speculate how far the rise to specific rank has been accelerated by isolation due to human interference. A similar phenomenon is shown by the genera *Pinus* and *Cedrus*.

The French authorities have created the forest of Mount Babor a National Park. As such it is protected from grazing by domestic animals. Barbey, however, makes additional constructive suggestions, maintaining that by the improvement of agricultural practice and by the establishment of communal forests open to grazing and for the provision of shelter and fuel, the natives will have no need which cannot be met outside the scheduled area.

The book gives a vivid impressionist account of the rural economy of the district round Babor, touches upon numerous biological problems of interest to the botanist, entomologist and forester, and shows the part which forest plays in the life of a community, aside from its usefulness in producing commercial timber. The book is profusely illustrated by excellent photographs.

A. S. WATT.

A Textbook of General Botany for Colleges and Universities. 3rd edn.

By RICHARD M. HOLMAN and WILFRID W. ROBBINS. 5½ × 9 in.

Pp. xiii + 626, with 1 plate and 426 text-figures. New York:

John Wiley and Sons; London: Chapman and Hall. 1934. 25s.

A Laboratory Guide for a Course in General Botany. By LEE BONAR, RICHARD M. HOLMAN and LUCILLE ROUSH. $5\frac{1}{2} \times 9$ in. Pp. vii + 112, with 1 text-figure. New York: John Wiley and Sons; London: Chapman and Hall. 3rd edn. 1934. 7s. 6d.

The first of these is a text-book which has reached a third edition in ten years and may therefore be taken as successful in meeting the requirements of American students. It covers rather more ground than an intermediate course in this country, and the mode of treatment does not differ in any marked fashion from that of English and continental books of similar scope. It is true that the authors aim at relating the subject to the practice and problems of agriculture where possible; but this aim is not obviously attained, except perhaps in the chapter on the fungi.

The first part of the book deals with the structure and physiology of seed-bearing plants. The chapters on root, stem, leaf, flower and seed are clear and orderly accounts. The introductory chapters on plant body and cell are less successful. Here the arrangement of the material is often clumsy, e.g. a few pages on movement are sandwiched between the classification of buds and a description of life cycles. The second part of the book consists of a survey of the plant kingdom. The chapters on the mosses, ferns and seed plants are good. The algae receive somewhat summary treatment. *Chlamydomonas*, so useful as an illustration of reproductive tendencies as well as from other points of view, is not used, no indication of the range of structure in the Siphonales is given; the importance of plankton is passed over. The fungi are more adequately dealt with and prominence is given to types of economic importance. It is doubtful whether the section on "Tendencies in the evolution of the flower" with its phylogenetic diagrams can be of much use in the absence of any description of representative families of flowering plants.

The final chapter deals with heredity and evolution and concludes with seven pages on fossil plants, which would have been better distributed through the systematic portion.

The illustrations are numerous and excellent. Great care has evidently been taken with their preparation. The photographs are well chosen and interesting. The diagrams—nearly all original—are pleasantly drawn and beautifully clear. They greatly increase the value of the book, which, taken altogether, is a very useful text-book.

The second volume, which is a laboratory companion to the text-book, gives detailed instructions for practical work. It should materially lighten the task of demonstrators and economise time in the laboratory. At the end of each section there is a batch of brief questions. Answering these may focus attention on important points, but the practice savours too much of the cramming system.

M. SKENE.

Identification of the Commercial Timbers of the United States. By H. P. BROWN and A. J. PANSHIN. $9 \times 5\frac{1}{2}$ in. Pp. xxvi + 164, with 274 photomicrographs. American Forestry Series, edited by WALTER MULFORD. New York and London: McGraw-Hill Book Co., Inc. 1934. 18s. net.

This new series has been planned to provide a co-ordinated set of text-books on the numerous subjects with which a professional forester is concerned in America. It is pointed out in the introduction to the series that American forestry is still in the pioneer stage and in need of both more exact and more scientific data and of a more scientific background for the development of indi-

vidual professional judgment, and it is to supply these needs that the preparation of this series has been undertaken.

The volume under review is essentially practical in its outlook and is concerned primarily with the scientific data necessary for the accurate identification of American commercial timbers, presented in such a form as to be of service to the teaching and practical forester, the plant anatomist, the engineer, the architect, etc. The book is not intended to cover the field of general wood anatomy or that of the properties and uses of wood, as these subjects will be dealt with in subsequent volumes. It is strictly limited to the identification of North American timbers, and the chief interest in the book therefore centres round the keys which are provided for this purpose.

There are two such keys, one based on features that can be seen with the unaided eye or with a hand lens and intended for practical use in the field, and the other, which involves the use of sections and a microscope, is more suited to the needs of the professional wood anatomist. The lens key is based primarily on anatomical features, but full use is made of subsidiary characters such as colour, taste, texture, etc. An attractive feature is the arrangement of the illustrations; a photomicrograph of the cross-section of each wood is provided opposite the appropriate page in the key, so that the illustration can be referred to without the necessity of turning over any pages. These photomicrographs are of the negative or direct print type, which by showing the pores dark suggests a solid end-surface as seen in the ordinary light; they are extremely clear and well reproduced, and although the magnification is low ($\times 5$), the fine details are fairly distinct.

The key based on the microscopic features calls for very little comment. Dimensions are fairly freely used, and care has been taken to explain how the figures have been arrived at. This key goes no further in the separation of species than the lens key, in spite of the greater number of features available and the greater precision with which they can be observed. Some indication of how particular species may be distinguished, is, however, given in the descriptions of the species following the keys. These specific descriptions include a fairly full account of the macroscopic anatomy and physical character of the wood and a rather brief but adequate survey of the microscopic anatomical features. Each concludes with a useful paragraph on the woods which are likely to be confused with the species being described and the best methods of distinguishing them.

The book is very well produced and is notable for the excellence of the photomicrographs with which it is illustrated and for the lavish scale on which they have been used.

L. CHALK.

Commercial Fertilisers; Their Sources and Use. By G. H. COLLINGS.
 $7\frac{1}{2} \times 5\frac{1}{2}$ in. Pp. xiv + 356, with 85 figures. Philadelphia:
 P. Blakiston's Son and Co. Price \$3.25.

This book has been prepared primarily for the benefit of students in the agricultural colleges of the United States, and is the result of long experience of investigation and teaching of the subjects it considers. The author also acknowledges much help from other experimental agronomists and commercial producers of fertilisers; the result is an eminently readable and informative exposition of the subject.

Of the book's utility to agricultural students this is not the place to speak, but general botanists, and especially those interested in the growth and nutrition of plants, will probably find a great deal to interest them. It has been, for example, one of the author's aims to explain where possible the reaction of plants to the nutrients commonly added to soils. This is admittedly difficult,

and probably no two experts could be fully satisfied by any one exposition. Most would probably agree, however, that Prof. Collings has shown sound judgment and restraint in his statements.

The book treats first of the early history of fertilisers and the founding of Rothamsted and other agricultural experimental stations. This is followed by an account of the Chilean nitrate industry and the manufacture of ammonium sulphate and the synthetic fertilisers containing nitrogen fixed from the air. The organic sources of nitrogen are mentioned next, and then the various sources of phosphates, and the manufacture of superphosphates and basic slag. The potash salts come next, and then a brief treatment of minor substances, such as sulphur, manganese, sodium and boron. The final chapters are devoted to the purchase and use of fertilisers in general.

English and Continental work, apart from a few references to the Rothamsted station, is little represented, but a good appreciation may be obtained of the investigations of the numerous stations in America, and of their contributions towards putting agricultural practice on a rational footing.

The illustrations are reproductions of photographs, and include views of factories and details of plants showing deficiency symptoms. A more systematic collection of the latter would probably be of greater use to the student than the numerous views of "treated" and "untreated" plots with human and other scale objects. The book is rounded off with a good bibliography and index.

W. O. JAMES.

Commercial Flower Forcing. By A. LAURIE and L. C. CHADWICK.
 $7\frac{1}{2} \times 5\frac{1}{2}$ in. Pp. x + 519, with 49 figures. Philadelphia:
 P. Blakiston's Son and Co. Price \$4.00.

Like the preceding book this seeks to place its subject on the firm basis of a rational knowledge of plants and of their structures and behaviour. This is mainly attempted in the second chapter which covers 18 pages. To outline the science of botany within such limits is a difficult task, and the authors have not been altogether successful in selecting the fundamental from the subsidiary. On p. 25 they have committed the error of suggesting that "the most important process in the absorption of water and solutes by the plant roots is that of osmosis".

In turning to topics such as greenhouse construction and management, vegetative propagation and the like, even the novice in horticulture can realise that the authors are on what is to them much firmer ground. Descriptions apply primarily, of course, to American conditions, and the sections on costs of production and wholesale marketing will not, perhaps, appeal much to English and other readers. On the other hand the accounts of photoperiodism, including much original work by the first author, the effects of watering plants, symptoms of various nutritional diseases, fungal and insect diseases and their control, and many others will have a direct appeal to the botanist of wide interests. Most of all, we fancy, the book will appeal to the botanist who possesses a small greenhouse, the detailed directions for the raising of numerous favourite plants being a great advance on the rule-of-thumb methods of the average gardening book.

W. O. JAMES.

An Introduction to Plant Biochemistry. By C. C. STEELE. Pp.
 viii + 356, with 12 figures. London: Bell and Sons. 1934. 15s.

Text-books that deal specifically with plant biochemistry are rare, and one that could be put into the hands of botany students with an experimental bias

has been an acutely felt want for a long time past. As this volume sets out to fill this very gap it is sure to receive a good deal of attention, appreciation and criticism.

The author describes all the categories of substances known to exist in plants, and, beginning with the simpler ones, builds up her descriptions in a manner that is logical and in accordance with the methods of organic chemistry. Metabolic considerations are for the most part separated into a final section being dealt with only when the chemical basis has been firmly established. While this last section cannot be regarded as comparable in standard with the earlier ones it is worth noting that it includes a concise account of the modern work on fruit ripening and storage (carried on especially by the Food Investigation Board), that is not readily accessible to botanists in other ways. The earlier chapters are sprinkled with directions for experimental work, mainly identifications, separations and occasional estimations.

The treatment is intended to be elementary; therefore considerable latitude has been taken in describing highly speculative or controversial topics. Cutinisation and alcoholic fermentation, for example, are rather inelastically dealt with. The physiological comments added to certain chapters will inevitably offend some tastes, and the very brief consideration given to pH is perhaps disappointing.

In all chapters the writing is clear and easy either for continuous reading or for reference and is thoroughly up to date. A bibliography of major references and a good index are supplied. In printing and binding the book is most attractively got up.

W. O. JAMES.

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CAMBIAL ACTIVITY, ROOT HABIT AND SUCKER SHOOT DEVELOPMENT IN TWO SPECIES OF POPLAR

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(With Plate IV and 6 figures in the text)

INTRODUCTION

IN 1930 Priestley⁽⁹⁾, as the result of an able review of the literature in conjunction with his own observations, suggested the general rule that in trees cambial activity recommences within the buds in the spring and spreads from there basipetally down the branches. This subject and its wider relationships were further discussed by the same investigator⁽¹⁰⁾ in his presidential address to Section K of the British Association for the Advancement of Science in 1932, and still more recently Priestley, Scott and Malins⁽¹¹⁾, using a new technique, claim to have confirmed this phenomenon in the case of thirty species of hardwoods and six species of softwoods.

Fewer observations are available on cambial activity in the root system of trees. Gulbe⁽⁴⁾, working with a group of trees which included a species of poplar (*Populus tremula*), found that it started either with the swelling of the buds or after the trees had leaved out and that it first became apparent in the young twigs during the first half of May, whence it spread basipetally down the trunk, reaching the thick proximal parts of the roots during the first half of June, and then continued into the thin distal parts of the roots during the second half of June. He also found that cambial activity ended in the same order, stopping first of all in the young twigs during the second half of August and ceasing in the distal parts of the root system during the second half of October, after which the roots remained completely dormant throughout the winter. Von Mohl⁽⁶⁾,

on the other hand, observed uninterrupted radial growth of roots during the winter but at a very much slower rate as compared with other seasons. These results were confirmed by Resa⁽¹²⁾ and Engler⁽³⁾ in the case of deciduous trees, but no activity was observed in conifers during the winter season. Recently Cockerham⁽²⁾ has observed slow continuous cambial activity throughout the year in the thin distal region of the roots of *Acer pseudoplatanus* upon which is superimposed, in the more proximal or middle region, the much more vigorous basipetal influence reaching it from the trunk. The dormant condition of the cambium, such as is found in the stem, was only observed in the roots for a short distance from the base of the trunk. Cambial activity was initiated in the new elongation growth within the bud and spread basipetally down the trunk and continued in the same direction into the root, as far as it went. He also found that cambial activity ceased in the same order. Another detailed study has been made by Wight⁽¹⁵⁾. He worked with specimens of *Pinus sylvestris* and observed a sudden resumption of radial growth uniformly along the trunk accompanied simultaneously by a slow basipetal spread down the branches from within the buds. He did, however, observe later, when about $\frac{1}{2}$ in. of new elongation growth had formed, that there was a falling gradient of xylem formation and lignification down the trunk. Cambial activity in the root did not become apparent until six weeks after its inception in the trunk, but again it was resumed uniformly along the length of the root. Cessation of cambial activity was found first of all at the root apices from which it proceeded backwards towards the proximal region of the roots. In the aerial parts of the tree the oldest branches ceased growth first, and the final cessation of growth took place in all parts of the branch about the same time. The uppermost branches, the whole of the trunk and the proximal parts of the root ceased growing last of all and at about the same time in all parts. These observations on *Pinus sylvestris* do not agree very well with Priestley's general statement, but Wight has admitted in his paper that, although he considered his conclusions justifiable, his data did not preclude the possibility of an exceedingly rapid basipetal "wave of initiation" of radial growth which spread down the trunk and continued in the same direction along the roots.

Various other investigators have observed elongation or extension growth of roots to continue far into the winter and in some cases throughout the winter, but there is no evidence of a causal relationship between elongation growth and radial growth in roots, such as

is known to exist between elongation growth and radial growth in stems. Wight (15) has examined the growing apices of roots of *Pinus sylvestris* in June and September and found no evidence that growth in length of the root had any effect upon radial growth, beyond a distance of 2 in. behind the new growth. Reference should be made to a paper by Stevens (14) for a useful summary of the literature on growth in roots.

MATERIAL

The observations recorded in this paper were made on trees growing in the vicinity of Edmonton, Alberta, Canada. In this region there are two native species of poplar, viz. *Populus tremuloides* Michx., the aspen, and *Populus balsamifera* L., the balsam poplar. The state of affairs in both these species was found to be identical; so henceforth the material will be referred to simply as poplar, but the bulk of the work was done with *Populus tremuloides* which is the more common of the two. The trees studied varied in age from one to twenty years. In this area, the usual and in all probability the only successful method of reproduction in poplar is by means of sucker shoots. Large quantities of viable seed are produced each year by the female trees, but successful seedlings rarely become established due to unfavourable environmental conditions. Suckering of poplar is particularly striking in certain areas of this province under certain conditions. The exceedingly rapid and uniform regeneration of poplar over burned areas, or in areas where the trees have been cut down in the process of clearing, is due entirely to the development of root suckers, with the exception of some suckers arising from the base of stems. When land which has been under cultivation is left undisturbed for any length of time progressive suckering from bordering poplar stands takes place very rapidly. Progressive suckering is also very strikingly apparent in the rolling park-land region, south-east of Edmonton, an area which has recently been described by Moss (7). On the north-facing slopes of the knolls there occur, commonly, groves of aspen poplar surrounded by typical prairie. These groves as such, in the area in mind, originated as root suckers, after a severe fire about twenty-five years ago. The innermost part of the stand consists of trees, more or less of equal age, the original sucker shoots in fact, now from twenty to twenty-five years old. Towards the margin of the stand the trees become on the whole progressively younger, and just beyond the margin there may be a zone of prairie varying in width from 10 to 20 ft. in which are to be found diffusely

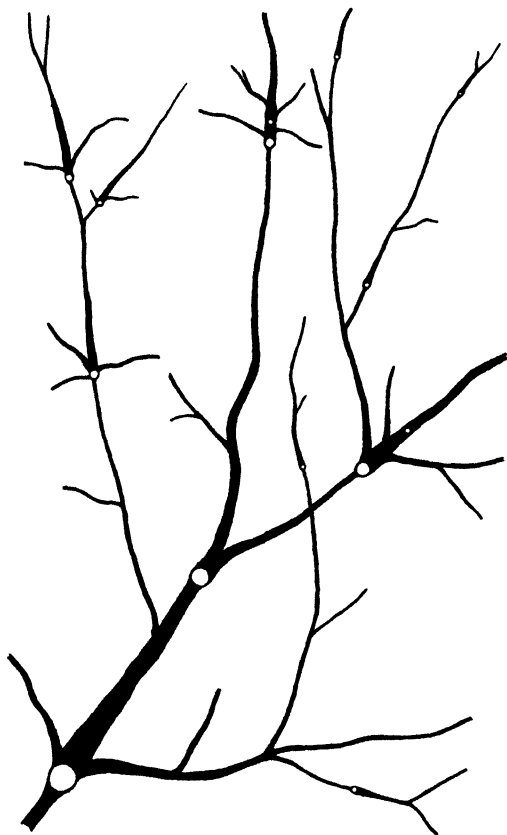
scattered poplar suckers, from one to five years old, spreading more or less radially outwards in the same centrifugal fashion with respect to age. Actually all the local poplar stands as they exist to-day have been derived from suckers, and it is for this reason that we find large numbers of trees on a common root system. The number of trees in a stand is usually greatly in excess of the number of true individuals present.

Most of the material studied was obtained from locations where poplar was spreading into unoccupied territory, for example grass-land or land which had been cleared of poplar and then left undisturbed. This type of material was selected because it could be more easily excavated than poplars growing in a close stand, and also because it was found to be more suitable for the observation of certain important points.

ROOT HABIT

The root system of the aspen poplar has already been described by Baker(1) and by Kittredge and Gevorkiantz(5), but these investigators do not mention certain important features which were very apparent in the material studied by the author. It is comprised of a shallow-seated, horizontally spreading system of scaffold roots upon which are borne the thinner fibrous feeder roots. For long distances the scaffold roots may be within a few inches of the surface of the soil, and the bulk of the underground parts is to be found in the first two feet of soil. Reference to Text-fig. 1 will illustrate the important features. The drawing is not made to scale, the length being reduced to a much greater extent than the width of the roots. This diagram might well represent a system, from 20 to 30 ft. long, in which the greatest root width is $1\frac{1}{2}$ in. and the smallest $\frac{1}{8}$ in., and in which the oldest shoot is about ten years and the youngest one year old. The sucker shoots are in section, the scaffold roots in surface view, whereas the fibrous feeding roots are not depicted at all. Attention should be paid to the following points. The sucker shoots borne on the young distal portions of the root system are the most recently formed, and on the whole they become progressively older as the older parts of the roots are approached. But it is not uncommon to find a young sucker shoot growing from a portion of the root system bearing much older shoots quite close by. Moreover, some roots run for long distances, up to 15 ft. for example, without giving rise to any sucker shoots at all and without varying appreciably in width throughout that length. What is perhaps the most striking feature of all in such

a root system is the fact that distal to the sucker shoots the root upon which the sucker arose is with few exceptions markedly thickened. This thickening becomes even more obvious if the tissues external to the wood are peeled off (Pl. IV, fig. 1). The grain of the wood can then be followed, and is found to run down that side of the shoot which is



Text-fig. 1. Scaffold root system of poplar showing progressive suckering. The sucker shoots are seen cut transversely and the scaffold roots in surface view.

distal and continues smoothly along the root on the distal side in the longitudinal direction. But on the proximal side the grain undergoes a sudden change in direction. In the region common to both root and shoot it divides and turns sharply through an angle of 90° to right and to left, after which the grain swings round and again runs longitudinally along that part of the root distal to the sucker shoot. This

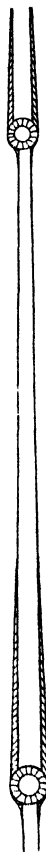
type of behaviour was found to be absolutely invariable under normal conditions. In a longitudinal radial section of the sucker shoot and that part of the root to which it is attached, the wood elements are all cut longitudinally or but slightly obliquely on the distal side, whereas on the proximal side, in that region common to shoot and root, they are cut completely transversely over a short distance (Text-fig. 5). Now if two suckers of approximately equal age arise close together on the same root, or if a younger sucker develops close but distal to an older sucker on the same root (Text-fig. 1), it is usually the case, so far as the more distal sucker is concerned, that that part of the root distal to it may not differ appreciably in thickness from that part of the root proximal to it, and for an obvious reason, viz. that the proximal part of the root is being thickened by the activity of the sucker shoot immediately proximal to it. This type of thing is found commonly in close even-aged stands. But examination of the grain of the wood in such cases will show that it behaves just as has been described above, i.e. it runs longitudinally down the shoot and continues longitudinally only along that part of the root distal to it. Another point of interest is that the development of sucker shoots often leads, after a time, to the production of new branch roots close to them, but invariably these new roots arise either from the base of the shoot itself or from that part of the parent root immediately distal to it.

THE INITIATION AND CESSATION OF CAMBIAL ACTIVITY

In this study xylem formation was adopted as the chief criterion of cambial activity. It was observed first of all, about the second week of May (1933), just below the expanding foliar buds, whence it spread basipetally down the shoot, and by the end of May a falling gradient of xylem formation and lignification had been established which in the case of young trees had reached the base, but in the case of some trees about twenty years old which were examined, xylem formation faded out some considerable distance above ground-level. Further study showed that this "flow" (a term to be discussed later) of cambial activity continued as such into the root system but only in a very definite direction, viz. along that part of the root distal to the shoot. An attempt has been made to depict this behaviour in Text-fig. 2, where the cross-hatching indicates newly formed xylem. It will be observed that from the base of every sucker shoot there has been established a falling gradient of xylem formation along that part of the root distal to the sucker, i.e. in the acropetal direction. It is also

true that immediately proximal to the shoot a falling gradient of xylem formation in the opposite direction was evident, but as shown in the diagram it was very feeble, of limited extent, and would soon be met by an acropetal "flow" from the first sucker shoot proximal to it. Material at the stage of development just described was found during the second half of June in the case of younger trees. No exception to this sequence of events was observed except locally, in the case of root injury, particularly grub injury, where cambial activity had been initiated at isolated points and quite independent of the acropetal "flow" from the shoots. It was found exceedingly difficult to obtain the ultimate growing points of the scaffold roots, but in many instances the roots were excavated until they were less than 1 mm. in diameter and no evidence was obtained of cambial activity, independent of the acropetal development from the base of the shoot. This was confirmed by examination of a few two year-old seedling trees in which the growing points of the roots were obtained. Here roots were found which for several inches behind the meristematic apex showed nothing but primary wood and the system as a whole, no indication of cambial activity independent of the stimulus emanating from the shoot.

As regards cessation of cambial activity, all the evidence pointed to the conclusion that it took place in exactly the same order as its inception. Examination of material of different ages from the middle of August onwards showed quite definitely that cambial activity ceased first of all in the youngest twigs and from there basipetally down the shoot into the root system, in which activity ceased in the same acropetal fashion as it started. The criteria adopted as indicating cessation of cambial activity were the fact that the tissues external to



Text-fig 2 Portion of scaffold root system of poplar bearing two sucker shoots, to show falling gradient of xylem formation. Cross-hatching indicates newly formed wood.

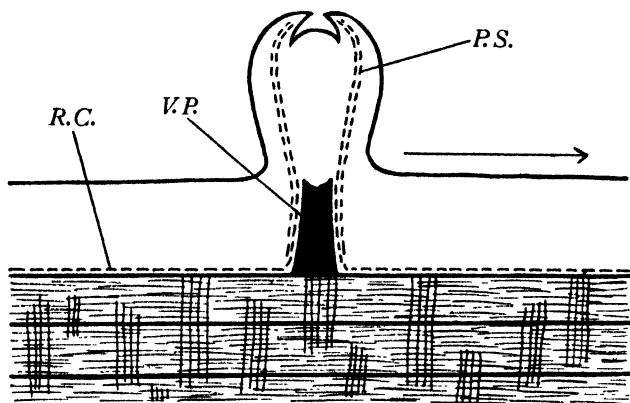
the cambium would no longer slip over the wood, and the presence of mature lignified xylem immediately adjacent to the cambium so that an even, clear-cut division between these two tissues was visible under the microscope. At the same time the appearance of the cambium itself was also studied before coming to any conclusion as to whether or not it had ceased dividing. It was difficult to decide just when cambial activity had ceased throughout the root system, but during the first half of September cambial activity could only be discovered in the thin distal portions of the roots where the diameter of the wood was not more than 0.5 mm. In view of Cockerham's(2) recent observation that cambial activity continues throughout the year in all but the more proximal parts of the roots of *Acer pseudo-platanus*, particular attention was paid to the appearance of the cambium in the roots of poplar during late autumn, but no differences were noted between the resting stem cambium and the root cambium at any point that would suggest anything but that the root cambium was dormant also.

There are certain items of interest worthy of mention at this point. As would be expected, it was found that cambial activity may have ceased in that part of the root distal to a sucker shoot, whereas in the older part of the root proximal to the shoot activity was still evident. However, observations were not so easily made of this type of thing as of the reverse, found when cambial activity was beginning, but this stage could be obtained, if pains were taken to secure at the right time material in which sucker shoots were located at a considerable distance from their nearest neighbours on the same root. It was found to be generally the case that the "flow" of cambial activity reached the roots earlier from younger than from older trees, and consequently the younger, more distal parts of the roots showed thickening before the older proximal parts of the same system bearing older suckers. In the same way the cambium had ceased to divide throughout the aerial parts of younger trees on the whole earlier than in older trees, with the result that in younger parts of the roots the cambium might be dormant, whereas in older more proximal parts of the same system it might still be active. Another point was noticed which at first proved to be rather confusing. It was not uncommon to find, in that part of the root immediately distal to a shoot, that growth was quite excentric, being more rapid on the lower side than the upper, and material was found in which growth appeared to have stopped on the upper side while it still continued on the lower side, whereas at points more distal and in the root proximal to the shoot cambial

activity had ceased both above and below. However, the explanation is probably quite simple. In the region of excentric growth cell division is exceedingly rapid and lignification lags behind, with the result that even after the cambium has ceased to divide several layers of immature, non-lignified wood elements may be found within it, thus giving rise on occasions to a localised region where the xylem merges gradually into the cambium, instead of being sharply delimited from it.

ORIGIN AND DEVELOPMENT OF THE SUCKER SHOOT

The sucker shoot is derived from a bud arising in the root phellogen, and since the phellogen, in the species worked with, proved to be pericyclic in origin, the sucker bud is really an endogenous structure. At an early stage in development there is a small nidus

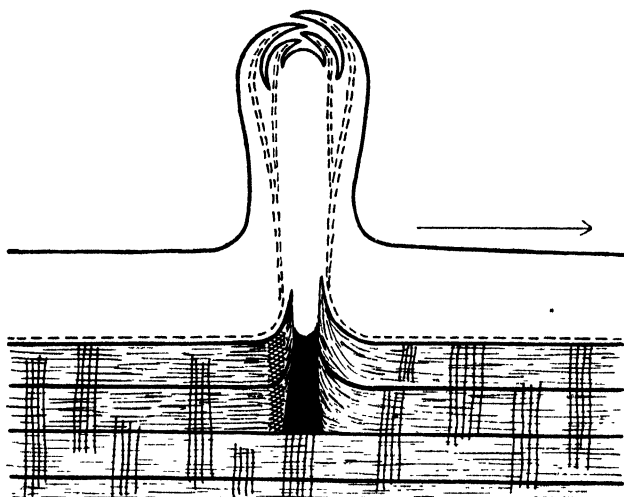


Text-fig. 3. Longitudinal radial section of sucker bud and root where vascular connection between bud and root has been made just as the root cambium became dormant. *P.S.* = procambial strand. *V.P.* = vascular peg. *R.C.* = root cambium. The arrow points in the distal direction.

of meristematic cells just within the cork and without any vascular connection whatsoever with the conducting system of the root. From this point development proceeds in two directions. Gradually a definite meristematic apex is established, giving rise in the usual way to enclosing leaf initials with their associated procambial strands, and at the same time vascular connection with the root is attained by the direct modification of a more or less cylindrical peg of bast cells, stretching from just below the bud to the central woody core of the root, to form pitted or scalariform tracheids. The ring of

procambial strands encloses this vascular peg and joins up below with the root cambium. This stage in development is depicted in Text-fig. 3, where vascular connection between bud and root has been made just as the root cambium became dormant. The bud and root are both cut in radial longitudinal section.

Text-fig. 4 illustrates the state of affairs two years later. The bud is now larger and more leaf initials have been formed, but in the interval two new rings of wood have been laid down by the root cambium. Notice first of all that this new wood encloses the vascular



Text-fig. 4. Longitudinal radial section of sucker bud and root, two years after the attainment of vascular connection between bud and root. Cross-hatching indicates xylem cut more or less transversely. The arrow points in the distal direction.

peg, and also that on the proximal side of the peg the wood elements suddenly become transverse, whereas on the distal side they are longitudinal at all points. Longitudinal sections which cut the vascular peg transversely and the root xylem tangentially (Pl. IV, figs. 2 and 3), give further information as to the orientation of the tissues at this stage of development. The picture now seen is that of a "flow pattern", essentially similar to that obtained when a liquid flows slowly round a cylindrical obstruction. Immediately proximal to the peg the wood elements turn sharply through an angle of practically 90° in the tangential plane, so that in radial longitudinal sections of the root these cells are cut transversely (Text-fig. 4), whereas on the

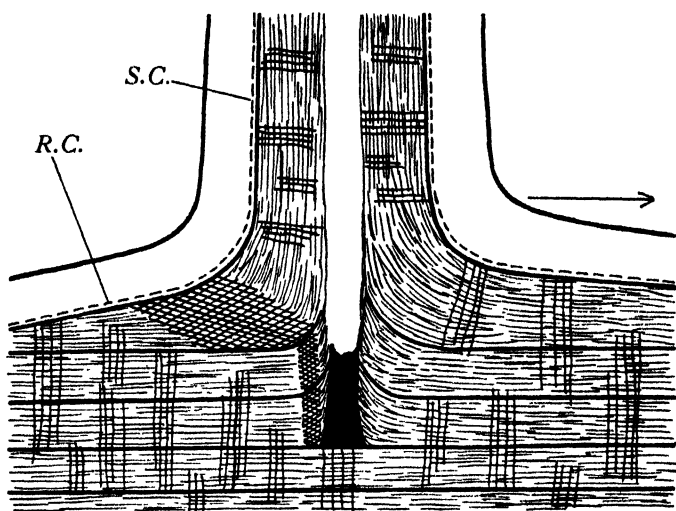
distal side the cells turn in again but slightly, so that they are cut almost completely longitudinally or at the most but slightly obliquely in longitudinal radial sections of the root. Sections through the phloem in the same plane reveal a similar state of affairs, and without a doubt this indicates the orientation to be found in the cambial layers themselves.

It should be remembered at this point that the vascular cambium of the root is continuous with the procambial strands in the sucker bud. By this time the procambial strands form a complete cylinder in which protophloem and protoxylem have begun to differentiate, so that cells common to both the root cambium and the bud cambium (ultimately the sucker shoot cambium) are involved in the reorientation of the plastic cambial layers on the proximal side of the bud.

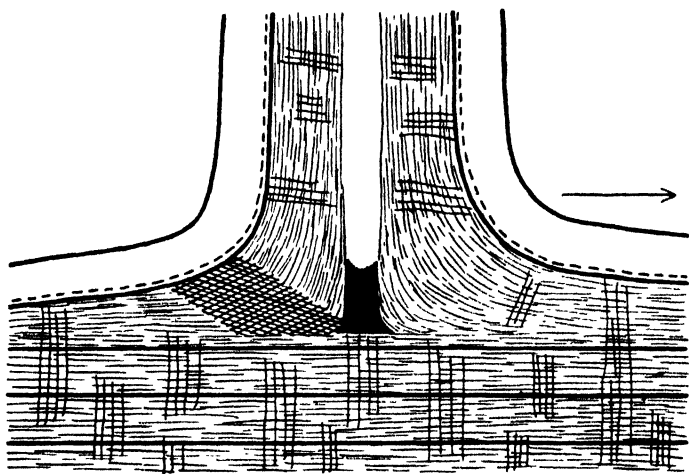
Moreover, it will be observed (Text-fig. 3) that a certain amount of xylem continuous with the root xylem has differentiated around the base of the bud. That this is really a manifestation of root cambial activity, rather than developmental activity of the bud itself, is shown clearly in cases where for some reason or other a sucker shoot has died, when it will be found that a similar wedge-shaped mass of wood is laid down around the base of the dead sucker shoot just where it joins the root.

Text-fig. 5 shows the final stage in development, in this case after another year has elapsed. During this last year the bud has gone ahead to form a shoot above ground, so that the drawing represents a radial longitudinal section of a one-year-old sucker shoot and the root upon which it arose. Notice again that the wood elements are cut transversely immediately proximal to the shoot, in that region common to both shoot and root, whereas elsewhere they are cut longitudinally. Another point that can be observed in the drawing is that the amount of wood laid down in the root distal to the sucker during the year is decidedly greater than that on the proximal side. The course of events in following years is a repetition exactly of what has already been described for the year in which the bud developed into a sucker shoot.

Sucker buds in all stages of development can be found on poplar roots at any time during the year. In the foregoing description of the development of the sucker bud a particular case has been selected, namely, where vascular connection between bud and root was completed just as the root cambium became dormant. It is important to note that this is not the only possibility, indeed it may happen but seldom. Actually, observation shows that vascular connection



Text-fig. 5. Longitudinal radial section of root and a one-year-old sucker shoot derived from the bud depicted in Text-fig. 4. *R.C.* = root cambium. *S.C.* = shoot cambium. Cross-hatching indicates xylem cut more or less transversely. The arrow points in the distal direction.



Text-fig. 6. Longitudinal radial section of root and a one-year-old sucker shoot, where vascular connection between bud and root was completed after cambial activity had recommenced in the root. This was followed by development of the bud into a shoot, during the same growing season. Cross-hatching indicates xylem cut more or less transversely. The arrow points in the distal direction.

between bud and root may be made at any time during the growing season, and that subsequent behaviour is along similar lines in all cases. The bud may not develop into a shoot for many years after vascular connection has been made with the root, but it is probably not quite true to say that the bud lies dormant, since it continues to form new leaf initials and on the whole increases in size. On the other hand, cases have been observed (Text-fig. 6) where vascular connection was completed after cambial activity had recommenced in the root, and development into a shoot followed during the same growing season. However, this latter type of behaviour does not appear to be common under normal conditions; usually, development is extended over a much longer period of time.

DISCUSSION

The foregoing observations have suggested to the author a conception of cambial activity in terms of a "flow". This idea of a cambial "flow" is based upon the following considerations: (1) the observation below the bud of "flow patterns" which presumably imply some type of flow mechanism for their formation; (2) the absence of anatomical evidence of any structural peculiarity of the cells, or of the architecture of the tissues that could account for the behaviour of the cells proximal to the sucker bud; (3) the fact that cambial activity in the region common to both root and bud is a manifestation of root cambial activity rather than developmental activity of the bud itself; and (4) the belief that the conception of a "flow" of cambial activity would seem to be a very convenient way of describing the observed behaviour in a more specific manner than, for example, "spread" of cambial activity.

Let us review in the light of this interpretation the example already described, where vascular connection between bud and root was made just as the root cambium became dormant. In the following spring, cambial activity spreads along the root in the acropetal direction from the base of growing sucker shoots. Now what does this acropetal spread of cambial activity involve? It means, firstly, that the cambial layers get progressively more plastic in this direction as the cell contents swell and are transformed from the gel to the sol state; secondly, that cell division follows in the same order; and thirdly, that differentiation and maturation of the xylem and phloem tissues take place in the same acropetal sequence. Just what happens when the vascular peg below the bud is encountered is beautifully illustrated by the "flow pattern" already described. At present no

attempt will be made to analyse the mechanism whereby the "flow pattern" is produced, beyond saying that the energy relationships within the plastic but structured cambial layers, or their still plastic derivative layers, are such that a reorientation takes place to form such a pattern. However, the attitude of the author is that reorientation of the cells to form the "flow pattern" is definitely a function of root cambial activity. Not only is it a function, but in some measure, at least, an expression of mechanism, interpreted in this paper in terms of a "flow" analogy. Observations of great importance in this connection are that vascular connection between bud and root may be made at any time during the growing season, and that subsequent behaviour is the same in all cases, resulting in the formation of a "flow pattern". On consideration, it is clear that these facts indicate that not only is cambial activity initiated as a "flow", but that it is maintained as such during its period of activity.

One possible objection to this conception of cambial "flow" will now be dealt with. Cambial activity in the shoots of plants is initiated within the developing buds, and it is quite probable that the sucker bud, despite its slow and prolonged development in many cases, would promote a certain amount of cambial activity below it, in its own axis, and perhaps even in the root axis. Now it may be that cambial activity emanating from the bud is governed by polarity, in this respect, that it can only develop in the morphologically distal direction in the root, and it is perhaps not inconceivable that polarity of this type would, in itself, be instrumental in the production of the "flow pattern" effect. However, any cambial activity on the part of the bud, in the region common to both bud and root where reorientation takes place, is completely masked by root cambial activity under normal conditions, particularly so if the root cambium is active when vascular connection is attained. It is extremely doubtful whether the bud exerts any influence at all in that region. Moreover, it is difficult to conceive of polarity in itself bringing about a reorientation of the cells to form the clearly defined "flow pattern" unless it were complete and unconditional, and of this there is no evidence. This point will be referred to again later in the discussion.

It is suggested that cambial activity may be expressed in terms of a "flow", but it still remains to explain the forces involved. At present no completely acceptable explanation, based on observational evidence, is forthcoming. Liquids flow from a point at which the pressure is higher to a point where the pressure is lower, and the pressure varies with the head of liquid. Obviously nothing completely

analogous to this takes place in the growing tree. There is definitely no actual movement of the plastic cambial initials from one point to another over any considerable distance. Nor is there any evidence of a basipetal or acropetal flow, in stems and roots respectively, of water from one cell to another, either in the cambium itself or in the differentiating layers cut off by it. Indeed, all the evidence points to the fact that movement of water is in the opposite direction, i.e. towards the apical growing points of the shoots. This means, of course, that the "flow pattern" is orientated in the opposite direction to the general movement of water in the plant. The author has given much thought to a consideration of the energy relationships of cambial "flow", but hesitates to theorise at present for lack of direct observational and experimental evidence. One possible explanation will, however, be indicated. The vessel is derived from a more or less linear series of cells which, according to Priestley (10), vacuolate progressively in the basipetal direction in the shoot and acropetally in the root. Vacuolation of the vessel segment involves entrance of water into the cell, accompanied by stretching of the cell wall, or in other words by an increase in turgor pressure, and the necessary water is derived from the previously formed layers of wood within, where the general movement of water is upwards. In effect, there is what may be termed a progressive "flow" of turgor or hydrostatic pressure within the developing vessel, in the opposite direction to the general movement of water. It is clear, of course, that this "flow" of turgor pressure would only be temporary in the formation of any one vessel, but the whole process is repeated in the development of every vessel, with the result that the "flow" of turgor pressure would be maintained over a long period of time in the same relative position, i.e. just within the actively dividing cambium. Further discussion along these lines is reserved, and it is simply suggested that here we may have a "flow" mechanism capable in one way or another of causing the formation of a "flow pattern" around the vascular peg below the sucker bud.

The conception of a "flow" of cambial activity could very readily be extended to explain in an extremely simple manner the relationship between a sucker shoot and the parent root. Briefly, the hypothesis is that reorientation of the cells, during development of the bud, in the region common to both root and bud on the proximal side, may predetermine the direction of "flow" of cambial activity emanating from the sucker shoot derived from that bud, or in other words, cambial "flow" from the shoot may be actually guided along

the root in the distal direction. This interpretation is along purely physical lines and does not invoke polarity of the type already mentioned when discussing the formation of the "flow pattern". But it does not of necessity preclude polarity, which although completely masked during the bud stage, might quite well manifest itself clearly after the bud has developed into an actively growing shoot. It is significant to note, however, that configuration of the tissues just proximal to the sucker shoot is anticipated in the bud stage. Moreover, polarity, assuming it to exist, is apparently not complete and unconditional, as witness the falling gradient of xylem formation on the *proximal* side of sucker shoots during the earlier part of the growing season. It would be interesting, in this connection, to isolate pieces of root system bearing only young buds which had not yet attained vascular connection with the parent root, and from which all shoots had been removed. However, such an experiment, simple as it is in theory, would be very difficult to carry out with poplar, as far as the author's knowledge of that material goes. But it is hoped that future work, of an experimental nature, will help towards a more complete understanding of some of the problems indicated in this paper.

Some recent experiments by SNOW⁽¹³⁾ have been interpreted as indicating that cambial activity is dependent upon the production of a hormone by the leaves. The present work neither confirms nor refutes this view, but it may be stated that in general, cambial behaviour in poplar is susceptible of interpretation in terms of production of such a hormone.

SUMMARY

1. Cambial activity, in the two species of poplar studied, is initiated within the buds, and spreads thence basipetally down the stem. From the base of the stem it continues acropetally along the roots. Cessation of cambial activity proceeds precisely in the same order as its inception.

2. Cambial activity begins during the first half of May, and the first indications of cessation can be observed during the second half of August. During the winter months the cambium remains dormant.

3. Root habit, and the origin and development of the sucker bud into a sucker shoot are described.

4. It is suggested, on the basis of a certain amount of evidence, that cambial activity may, in a general way, be interpreted in terms of a "flow".

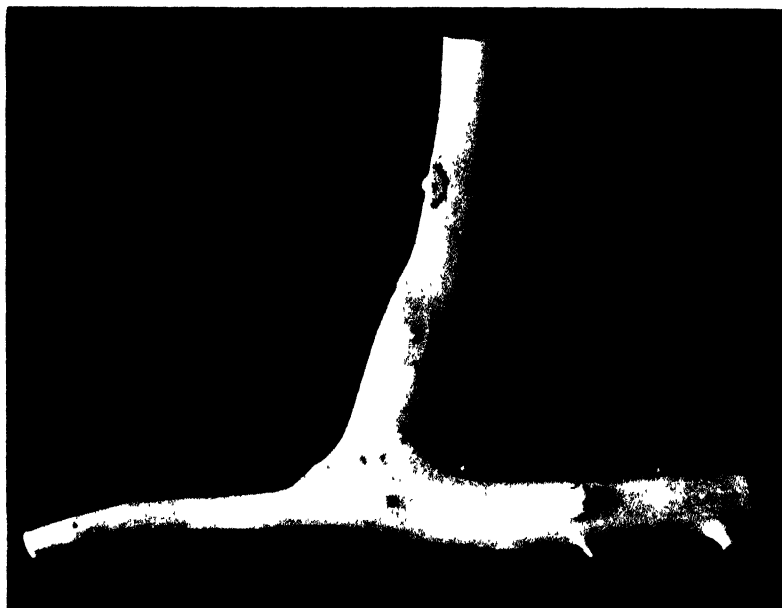


Fig 1



Fig 2



Fig 3

BROWN—CAMBIAL ACTIVITY IN TWO SPECIES OF POPLAR

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EXPLANATION OF PLATE IV

- Fig. 1. Four-year-old sucker shoot on eight-year-old root, showing grain of wood and distal thickening of root. Natural size.
- Fig. 2. Section, longitudinal tangential of root and transverse of vascular peg below the sucker bud to show flow pattern effect $\times 15$.
- Fig. 3. As Fig. 2. A different preparation more highly magnified. $\times 45$.
- In Figs. 2 and 3 the tissues above and below the vascular peg are respectively proximal and distal.

OBSERVATIONS ON RESISTANCE TO
POWDERY MILDEWSBy E. J. H. CORNER
Botanic Gardens, Singapore

(With 2 figures in the text)

As little seemed to be known of the way in which certain varieties of a host species resist the attack of mildew, I experimented with *Erysiphe graminis*, *Sphaerotheca pannosa* and *Podosphaera leucotricha*, determining first the course of infection on susceptible varieties, then on the resistant and finally on a miscellany of inappropriate hosts. The results show that, under suitable conditions and provided that the cuticle is not too thick, mildew conidia will germinate on any plant up to the stage of penetration of the cellulose layer of the host cell and of formation of an infection papilla, but that except on susceptible varieties the penetration process is then killed before it can enter the cytoplasm or after it has formed a rudimentary haustorium. I examined with particular care the exact method of penetration of the host cell, and certain points of interest in the germination of the conidia have come to light. Zimmermann (30) in 1924 summarised all earlier work on the powdery mildews.

METHODS

Leaves were inoculated by placing fresh conidia on marked areas. Rose and apple leaves were placed on damp filter-paper in Petri dishes; wheat and barley leaves were left attached to the plants, and these were covered with bell-jars. The pieces of leaf were fixed in 1 per cent. chromacetic solution and sections (3-5 μ thick) were stained, diamant fuchsin and light green being the most satisfactory combination. Alternatively, the epidermis was stripped off and stained with cotton blue and lactophenol in order to follow the germination of the conidia, or it was subjected to microchemical tests to determine the effect of the haustorial process on the wall of the host cell. The method of stripping the epidermis proving the more satisfactory from its readiness and simplicity, I generally inoculated the underside of leaves.

ERYSIPHE GRAMINIS

Material. Wilhelmina wheat, Spratt Archer barley and *Agropyron repens* were taken as susceptible plants, each having its physiologic form of mildew, and Norka and Persian Black as resistant varieties of wheat. The plants were grown in pots in a greenhouse. The susceptible varieties, becoming spontaneously infected, served for the supply of conidia.

Thin sections of the cuticle and epidermis of such leaves are difficult to prepare: silicification hardens the cuticle, causing it to be fractured rather than sliced by the microtome. Material for cytological purposes was therefore dehydrated in glycerine and cleared in cedar-wood oil, to obviate further hardening in alcohol and xylol, and embedded in paraffin of high melting-point. Hydrofluoric acid softens the cuticle without apparently affecting the soft tissues, but the discovery came too late to be of use.

Germination on susceptible varieties. The conidia germinate by producing from near one end a straight or somewhat flexuous, clavate, *primary* germ tube, $20-40\mu$ long $\times 4.5-6\mu$ wide at the apex $\times 3\mu$ wide at the base. It is cut off by a septum near the conidium and it proceeds directly to form a haustorium either from the immediate underside at $1-3\mu$ from the apex, or from the apex itself, if pressed into the groove between two epidermal cells, or from a small sub-apical appressorium, as on the vegetative hyphae. After this first haustorium has been established, the germ tube continues its apical growth as an ordinary hypha. The original "germ tube portion" becomes the first cell and haustoria subsequently arise from the third, fourth or fifth cells, rarely if ever from the second. At the same time $2-4$ *secondary* germ tubes arise from near the ends of the conidium: they grow directly into hyphae and produce haustoria from their third or fourth cells, rarely from the second and apparently never from the first. Furthermore, *tertiary* germ tubes may develop. They are short, tapered processes, $4-10 \times 1-3\mu$, arising from any part of the spore, and generally abortive. Old conidia may develop only tertiary germ tubes, and some of them may attempt to form haustoria without success. Two to five laterals subsequently arise from the cell which was the primary germ tube.

On all three susceptible varieties the germination of fresh conidia always took this precise course, which follows, evidently, from the fact that the conidia do not contain sufficient reserves to produce directly a mycelium but, in spite of their large size, must needs draw

upon the host at the earliest opportunity. The conidia are swollen with water, having highly vacuolate cytoplasm unlike the dense contents of most spores, and this, as will be explained later, supplies the water of germination.

After 24 hours from the sowing of the conidia, at a temperature of *ca.* 20° C., and in a saturated atmosphere, penetration of the host cell is just beginning. After 48 hours, the first haustoria are more or less fully grown, and one or two secondary germ tubes may have arisen. After 72 hours, all the secondary germ tubes have developed and a fairly extensive mycelium has formed, the hyphae being 200–300 μ long (Fig. 2); the secondary haustoria are as yet immature. After 96 hours, conidial chains are formed.

Penetration of the host cell. The cell wall is penetrated and the haustorium develops in the way described by Grant Smith⁽²⁸⁾ and Foëx⁽⁹⁾. A stylar process from the germ tube pierces the cuticle, and in its passage through the cellulose layer it is preceded by a local thickening of the layer into a papilla which it eventually pierces at the apex (Fig. 1). The process sticks like a spine into the cell wall; it becomes conical with rather a wide base, 1–1.5 μ , but the base appears to narrow again as the apex pushes into the papilla. Inside the host cell it dilates into the haustorium and in doing so it invaginates the lining layer of cytoplasm. The haustorium develops as a small pyriform body which becomes drawn out along the long axis of the host cell, and from the ends the finger-shaped appendages arise; the whole gradually enlarges into the mature organ which is 50–90 μ long, with the body 16–25 \times 7–10 μ . Ultimately, according to Grant Smith, the appendages may poke through the lining layer into the cytoplasm itself. There is no obvious interaction between the nucleus of the host cell and the haustorium. Grant Smith observed no action in the case of *Erysiphe communis* on *Geranium*.

When the conidia are sown thickly, several haustoria may develop in one cell. According to Foëx⁽⁹⁾ the haustoria are then much smaller than when a single haustorium is present in a cell.

Microchemical tests. In epidermal strips stained with cotton blue, there is generally a blue halo, 10–25 μ wide, round the point of penetration. In strips stained with Schultz's solution, colourless circles with a colourless papilla in the centre are to be seen on a field of purple (the unaltered cellulose of the cell walls). But neither of these methods is reliable, and Schultz's solution, if allowed to act for long, causes the cellulose to break up into jagged purple strips transverse to the long axis of the cell and separated by wide colourless areas.

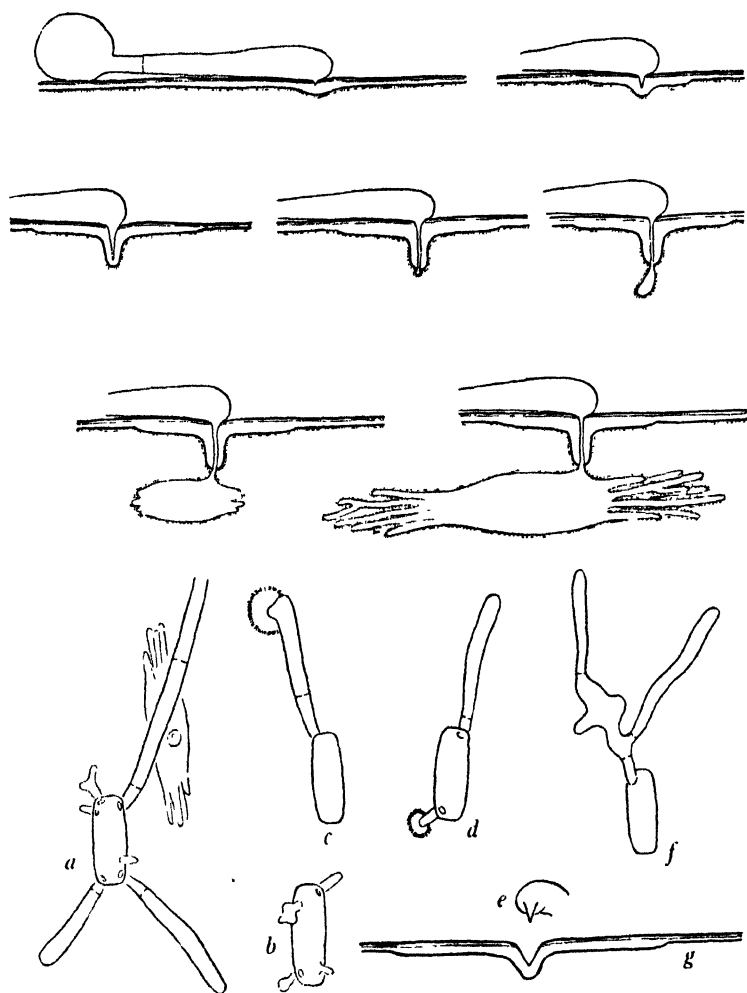


Fig. 1 *Erysiphe graminis*. Above: seven stages in the development of the haustorium, $\times 1000$. Below: a, a fully germinated conidium with a primary, two secondary, and three tertiary germ tubes, $\times 500$; b, an old conidium with four tertiary germ tubes, $\times 500$; c, a conidium with a primary germ tube and a "cotton-blue halo", $\times 500$; d, a conidium germinated on *Polypodium aureum* with a "cotton-blue halo" beneath a tertiary germ tube, $\times 500$; e, the tip of a germ tube with its penetration process, $\times 1000$; f, a conidium germinated on a begonia leaf, $\times 500$; g, an infection papilla in section with the germ tube detached, $\times 2000$.

A better method is to soak the strips in iodine solution for 4–5 min., transfer to 66 per cent. sulphuric acid for 5–10 min., then wash and mount in dilute glycerine. Colourless, or yellowish, circular patches, 10–25 μ wide, each with a colourless papilla in the centre, are then seen on a background of deep blue. The edges of the circles are blurred.

The colourless circles arise as minute areas, 1–2 μ wide, beneath the tips of the germ tubes before there is any sign of a papilla, although even in the smallest circles a dark spot can always be detected, which is where the penetration process pierced the cuticle. As the circle widens the papilla develops. An enzyme evidently diffuses from the process and alters the cellulose layer of the host cell wall. The altered part is distinctly swollen—it is about twice as thick as the unaltered layer—and it is probably the intense local action around the penetration process and the thrust of the process which make the papilla. Cotton blue stains only the part of the wall which is being altered, that is the circumference and papilla, so that small blue circles with dark centres in the initial stages of penetration develop into the blue haloes. The altered part stains also with stains such as haematoxylin, gentian violet, safranin and diamant fuchsin.

Although the cellulose is so clearly altered round the point of penetration, the cuticle is unaffected. In microtome sections it appears as a hyaline strip, neither swollen nor stained in any peculiar way, above the altered cellulose. In epidermal strips treated with Schultz's solution or iodine and sulphuric acid, it stains yellow whether over the altered or unaltered parts of the wall: and it does not adsorb cotton blue or nuclear stains, which it surely would do if it suffered decomposition. The cuticle must be penetrated mechanically. The tip of the germ tube is pressed very closely to the epidermis, often causing a slight yet distinct depression of the cell wall, but it has no obvious means of fixation: no mucilage sheath was disclosed by indian ink. But, as Brown and Harvey⁽⁵⁾ have shown, the germ tube requires merely to be adhesive in a narrow ring round the point of penetration.

Germination on Norka wheat. Germination proceeded normally for 24–36 hours up to the formation of the infection papilla. There, in most cases, the penetration process was stopped and the conidium and germ tube died without further development. There were always a few cases, however, in which the penetration process gave rise to a small haustorium, with or without rudimentary appendages, and able to absorb sufficient nutriment to allow one or two secondary germ tubes to develop and produce similar haustoria. Such mycelia

grew slowly, being composed of only 4-8 cells after 72 hours (Fig. 2), and in five or six days they died. Occasionally a conidium gave rise to two germ tubes, each of 2-3 cells, which produced one or two papillae without haustoria. In three cases a normal mycelium with full-sized haustoria developed exactly as on the susceptible varieties, and conidia were produced after 120 hours. I did not experiment with these conidia.

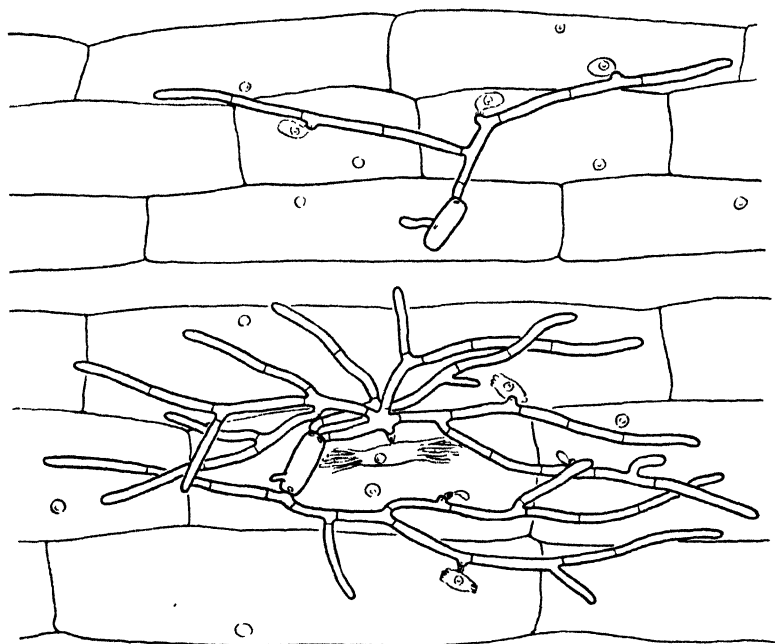


Fig 2 *Erysiphe graminis*. Mycelia 72 hours old. Above, on Norka wheat; below, on Wilhelmina wheat: $\times 300$.

Evidently Norka is not entirely resistant. Attempts were made to infect the glumes, as being possibly more susceptible organs, but without success: penetration stopped at the papilla stage or, as frequently, there was no penetration at all, which might have been due to the thickness of the cuticle. It should be mentioned that to avoid such a contingency in these experiments the basal part of half-grown leaves was inoculated.

Germination on Persian Black wheat. As on Norka, germination proceeded to the papilla stage, but neither haustoria nor secondary germ tubes were produced; the conidia gradually died. Evidently

the penetration process is killed before it can enter the host cell, and Persian Black is completely resistant.

Scars. Some leaves of both resistant varieties were examined four weeks after inoculation. Colourless circles, each with a papilla in the centre, were as plain as if new, and these marks of attack persist probably throughout the life of the infected part. The circles were too numerous to have been caused by casual infection.

Cross inoculations. Conidia from Spratt Archer barley and *Agropyron repens* placed on Wilhelmina, Norka and Persian Black wheat germinated up to the papilla stage without producing haustoria. Similar results were obtained with conidia from Wilhelmina wheat placed on Spratt Archer barley and *A. repens*, and from *A. repens* on Spratt Archer barley. Salmon⁽²²⁾ obtained similar results, observing that, after 24-36 hours, rudimentary haustoria were generally developed and sometimes a scanty mycelium which invariably degenerated in 5-6 days.

PODOSPHAERA LEUCOTRICHA

Material. Cox's Orange Pippin, Stirling Castle and Bramley Seedling apple trees were taken as susceptible varieties, Worcester Pearmain as resistant.

Methods. The plants were grown in a greenhouse; the susceptible varieties became spontaneously infected and provided supplies of conidia. As it was necessary to inoculate the upperside of the leaves on account of the tomentum underneath, the strip method was impracticable; the mesophyll cells could not be cleared away sufficiently to render the epidermis transparent. The germination of the conidia was followed either by clearing the pieces of leaf in acetic alcohol (1: 2) or after staining with cotton blue and mounting whole.

Germination on susceptible varieties. As with *Erysiphe graminis*, the conidia produce a primary germ tube from the end of which the first haustorium arises, but only one secondary germ tube; the germ tubes grow from any part of the conidium. Penetration and formation of an infection papilla occur as in *E. graminis*. The papilla and the surrounding part of the cellulose layer, which is distinctly swollen, stain intensely with nuclear stains. The cuticle remains unaltered. The primary germ tube measures $25-40 \times 4.5-5 \mu$ at the apex, $3-4.5 \mu$ at the base: it develops within 24 hours and the primary haustorium within 48 hours.

Apparently a mycelium cannot be established on the upperside

of mature leaves on account of the thickness of the cuticle. The conidia formed germ tubes, but I saw no sign of penetration.

These observations agree essentially with those of Woodward (28).

Germination on Worcester Pearmain apple leaves. This variety is not entirely resistant. A few opening buds were fully infected, the mycelium developing conidia. Half-grown or mature leaves were never infected, but in one instance a cluster of flowers was mildewed. I did not examine plants in the open.

Conidia germinated on young leaves up to the papilla stage and there infection generally stopped. Rarely, a normal mycelium developed and produced conidia in a week's time.

SPHAEROTHECA PANNOSA

Material. Dorothy Perkins and Crimson Rambler roses were taken as susceptible varieties, Gloire de Dijon and American Pillar as resistant. The plants were grown in pots in a greenhouse.

Methods. The same methods were used as with apple mildew. A satisfactory fixative not having been found, the details of penetration could not be made out. Chromacetic solution caused strong contraction of the radial walls and buckling of the outer wall of epidermal cells, while acetic alcohol (1 : 3) and Bouin's fluid, though causing less contraction, rendered staining difficult. The epidermis did not strip easily.

Germination on susceptible varieties. At a temperature of *ca.* 20° C. the conidia germinate rapidly. The primary germ tube and haustorium are formed in 24 hours, and in 48 hours 2-3 secondary germ tubes have arisen and produced a fairly extensive mycelium with young conidiophores of 4-5 cells; in 60 hours some conidia have matured. The germ tubes arise from each corner of the conidium, giving it a cruciform appearance. The mycelium grows rapidly on young leaves but dies on mature leaves, especially those of Dorothy Perkins, which is probably due to the thickening of the cuticle. Germination occurs readily on either side of the leaf, all the stomata being on the under-side in these varieties of rose.

Microchemical tests showed a distinct alteration of the cellulose layer round the point of penetration. Staining with Schultz's solution and iodine and sulphuric acid showed colourless patches as with *Erysiphe graminis*.

Observations on resistant varieties. Germination proceeded to the papilla stage and there, in general, it stopped. With Gloire de Dijon germination was occasionally normal and conidial chains were pro-

duced after 60 hours. Furthermore, in the greenhouse, young leaves of this variety were commonly mildewed, so that it is but partly resistant, like the Worcester Pearmain apple variety. With American Pillar a few conidia managed to form a scanty mycelium, derived from 1-3 germ tubes, but it invariably died in a week without sporangia. American Pillar was never seen to be mildewed, so that at most it is slightly susceptible, like Norka wheat. Under normal conditions both of these rose varieties are as resistant as Persian Black wheat.

I tried several times to infect the petals of Gloire de Dijon and the wild *Rosa canina*, but there was never a sign of penetration although germ tubes developed. The cuticle on the petals was remarkably thick, separating as a yellow pellicle on treatment with iodine and sulphuric acid.

CONCLUSIONS ON PENETRATION

The following steps can be distinguished in the penetration of the host cell by the haustorial process.

The stimulus to penetration. The tip of the germ tube and the appressoria must respond thigmotropically to contact with the epidermis. As yet, there is no evidence for positive chemotropism. As will be shown, the germ tubes will attempt to penetrate a great variety of unrelated plants, and Neger(16) found that they would form appressoria on contact with a hard surface. Foëx(9) has illustrated a conidium which has formed a characteristic appressorium on another. The germ tubes are undoubtedly thigmotropic.

Penetration of the cuticle. This is evidently mechanical, neither physical nor chemical alteration being noticeable about the point of penetration. A thick cuticle prevents penetration. Woodward(28), on the other hand, considered that it was in part chemical in the case of *Podosphaera leucotricha*, chiefly because the penetration process was slightly swollen in the cuticular layer; his figures suggest rather that the swelling occurred at the junction of the cellulose and cuticle.

Penetration of the cellulose layer. This step is clearly both mechanical and chemical. Yet, if the cuticle is pierced by pressure, why not the cellulose? Is it so much thicker and denser that it should necessitate swelling and softening, or is the production of pectinase but a relic of a former state of endoparasitism? It may be that by chemical alteration of this layer mildews avoid the necessity of deforming it, which step Brown and Harvey(5) state to be the most difficult in mechanical penetration.

Grant Smith⁽²⁶⁾ and Woodward⁽²⁸⁾ considered that the infection papilla might be formed by deposition from the host cell as a means of resistance against the parasite. If that were so, one would expect that the nucleus would be concerned, that the cytoplasm would stain deeply about the papilla and be firmly adherent to it, that the thickened part of the cell wall and the papilla would be stratified, and that not merely the base but the whole haustorium would be encased in cellulose. There are no such indications. On the contrary, the swelling and alteration of the cellulose layer in a circular patch of bleary aspect and blurred outline, having the point of penetration as the centre and the most intense action round the penetration process, conform exactly with effects to be expected from the diffusion of a cytase from the process. The secretion of cytase evidently continues after the haustorium is developed, for the papilla becomes partially dissolved and dwindles to a low collar about the stalk. Experiments with spores on strips of dead epidermis would prove the point.

Woodward⁽²⁸⁾ states that with *Podosphaera leucotricha* the cellulose layer may be acted upon by diffusion from the hypha before the cuticle is pierced. In the case of *Erysiphe graminis*, I never observed alteration of the layer in strips stained with iodine and sulphuric acid without there being a dark point in the centre of the patch indicating penetration; it was necessary to examine the strips with an immersion lens, as the penetration process is at first exceedingly fine. With rose petals the cuticle was not pierced nor was the cellulose layer below the germ tube in any way affected.

Intrusion of the haustorium. Unless the turgor pressure in the penetration process is greater than that in the host cell, the process could not swell into a haustorium without piercing or killing the cytoplasm, which it appears unable to do. That turgor pressure may be an important factor in resistance has been shown by Hawkins and Harvey⁽¹¹⁾ in experiments with *Pythium de Baryanum* on potato tubers: certain resistant varieties of tuber were found to have a higher pressure in the cell vacuole than the parasite. Moreover, it is known that plants in a flaccid state tend to be more susceptible to mildew: Rivera⁽¹⁹⁾ and Volk⁽²⁹⁾ have studied this problem in connection with *Erysiphe graminis*. But the flaccid state may assist rather the entrance of the penetration process by facilitating the deformation of the cell wall and the development of a steep-angled cone of penetration⁽⁵⁾.

These results on the penetration of the epidermis by mildews

agree essentially with those obtained by former investigators with *Botrytis cinerea*(1), *Colletotrichum lindemuthianum*(8), *Sclerotinia liberiana*(2), and with the basidiospores of *Puccinia graminis*(27). They conform also with the theory of mechanical penetration of the epidermis put forward by Brown and Harvey(5). Considering how different these fungi are, one is led to suppose that such is the general method of entry by parasites which pierce straight into the host and that it may not be as difficult as one would at first imagine. The infection papilla is characteristic, however, of mildews, with the single exception of the bryophilous discomycete *Neotiella crozalsiana*(7).

CONCLUSIONS ON RESISTANCE

Each stage in penetration may be opposed by a barrier. Too thick a cuticle or cellulose layer or a high turgor pressure may debar the haustorial process, and on entry into the host cell it may encounter toxins. That the process should be stopped at the papilla stage in all cases of true resistance, after overcoming the obstacles in the cell wall, suggests that in the main toxins are the basis of resistance. From the point of view of the host plant the means of resistance is highly satisfactory; the parasite never obtains a footing in its tissue and there is no question of a prolonged struggle or the construction of an expensive barrier of dead cells.

As regards the chemical nature of resistance, Marañón(13) has put forward an ingenious hypothesis based on his analysis of the leaves of varieties and hybrids of *Oenothera*, susceptible and resistant to *Erysiphe polygoni*. Resistance is correlated with an appreciably higher tannin content and water-soluble acid content, and it is suggested that the tannin kills the fungus, being a protoplasmic coagulant to which many fungi are susceptible, and that the acid prevents the action of the tannin on the cytoplasm of the host. It remains to show how the hyphae of mildews react to different concentrations of tannin. On such a basis of quantitative differences in the toxin, one can readily explain the partial infection and occasional full infection of resistant varieties of a host species, such as happens in the case of Norka wheat, Worcester Pearmain apple or Gloire de Dijon rose, but to be of general application the hypothesis must needs be qualified *ad extremum*. Mere quantitative differences, no matter what the toxin, could never account for the sum total of resistance to mildews when the high specific infectivity of the whole family is considered. Those very varieties of *Oenothera*, for example, which are susceptible to that physiologic form of *Erysiphe polygoni* are at the

same time resistant to every other species of mildew, and yet that physiologic form of *E. polygoni* cannot infect one of the hosts of the other mildews. There must be qualitative differences in the toxins by which different species of host plant resist different physiologic forms of mildew. And one may doubt, indeed, whether by bulk analysis such differences could be detected or even the nature of the toxin.

Salmon has shown that when experimenting with biologic species (physiologic forms) one must beware of a subinfection of resistant hosts caused by a heavy inoculum. If several germ tubes attempt to enter one epidermal cell, that cell may be unable to resist so many, and one or more may develop haustoria and a scanty mycelium, which soon dies, however, with or without sporing. Such may have been the case in some of my observations on the partial resistance of Norka wheat, Worcester Pearmain apple and Gloire de Dijon rose; in others, as shown in Text-fig. 2, one conidium was certainly able of itself to invade a host cell and even develop a normal mycelium. These varieties may fairly be judged imperfectly resistant in contrast with Persian Black wheat, which was never infected, though subjected to equally heavy inoculation. American Pillar rose occupies an intermediate position, for what scant mycelia it might support were sterile. Proof of the nature of subinfections must lie in inoculation with the spores, which should show whether a new and more virile strain had arisen, as that of the wheat mildew which Salmon⁽²³⁾ educated on to barley leaves. On the other hand, with *E. polygoni* on cultivated Brassicae, Searle⁽²⁵⁾ considered that the fungus over-wintered by means of autumnal subinfections, which developed fully in the following spring.

So varied may be the degrees of infection caused by a mildew, not merely on different host species or varieties, but even on exactly similar plants of the same species, wild or cultivated, that it is impossible at present to arrive at any conclusion concerning the nature of parasitic specialisation in the family. Many are the records of anomalous infection. With *Erysiphe cichoracearum* Neger⁽¹⁷⁾ has described from the same host species instances of full infection, subinfection and no infection, as determined microscopically; conidia from *Sonchus asper* caused subinfection on *S. oleraceus* in summer and full infection in autumn, likewise the conidia of *Sphaerotheca humuli* from *Epilobium montanum* on *Taraxacum officinale*. In such cases the inhibitory effect of high temperature must be taken into consideration. Similarly, with *Erysiphe polygoni*, Searle⁽²⁵⁾ found that

conidia from the swede might infect plants of *Brassica sinapis* normally or not at all. Most remarkable are some of Neger's early experiments (16). A mildewed plant of *Spiraea ulmaria* was grown for three weeks under a bell-jar in contact with a healthy plant and, whereas the leaves of the mildewed plant were spontaneously infected as they unfolded, the healthy plant was not once attacked: so, too, with mildewed and healthy plants of *Ranunculus repens* and of *Plantago major*. Biologic specialisation seeming to be much less strict in the mildews than in rusts, one could wish for inoculation experiments with pure strains of the fungus derived from single spores, conducted under comparable conditions and with a microscopical examination of the extent of infection.

It appears that other hosts may have a rather different, if less effective, method of resistance from what I have described. Neger (17) found, in the cases of subinfection caused by *Erysiphe cichoracearum*, that the rudimentary haustorium from the primary germ tube was entrapped by a dark brown, gummy substance which was deposited round it on entry into the host cell; the haustorium was killed but the host cell also died. Woodward (28) found a similar occlusion of the haustoria of *Podosphaera leucotricha* in connection with the browning of apple leaves; browning of the leaf cells occurred in the immediate vicinity of the occluded haustoria, the extent of browning varying with the number of haustoria, so suggesting the escape of toxic substances from them.

If the stimulus to penetration rests with the thigmotropism of the germ tube, conidia must attempt to penetrate any solid object on which they can germinate. I experimented therefore with a number of inappropriate hosts, using the strip method of examination, to discover to what extent they might be infected.

EXPERIMENTS WITH INAPPROPRIATE HOSTS

Erysiphe graminis on *Impatiens Sultani*. A primary germ tube developed in 24 hours and gave rise to an infection papilla by penetrating the cuticle and cellulose layer in the normal way. There was no further development, and I could see that the stylar process from the germ tube just reached the tip of the papilla. Cotton blue gave a small blue halo, Schultz's solution and iodine and sulphuric acid a small colourless circle, round the point of penetration. Some processes were apparently unable to penetrate, and the best marked cases of alteration of the cellulose layer were on the elongated cells overlying the veins of the leaf: there the colourless patches were

10–20 μ wide. Grant Smith⁽²⁶⁾ found in *Phyllactinia coryleae* that the hyphae entering the stomata generally grew straight towards a vascular bundle if there was one in the immediate neighbourhood, forming large haustoria in the cells of the bundle sheath: and Salmon observed the same tendency in *Erysiphe graminis* when induced to grow endophytically from wounds⁽²⁴⁾.

Erysiphe graminis on *Cobaea scandens*. Infection was stopped at the same stage as on *Impatiens*, but the stylar process, which was very fine indeed, penetrated in fewer cases, and the papilla and altered area of cellulose were much smaller.

Erysiphe graminis on apple, rose, broad bean, hyacinth, tulip, and begonia. A primary germ tube developed with the tip closely pressed to the leaf, but there was no sign of a papilla or of zoning on treatment with cotton blue. These experiments were performed before I realised the uncertainty of staining with cotton blue and should be repeated with iodine and sulphuric acid. On apple and begonia leaves the germ tube became strongly lobed and distorted after 48 hours, and gave rise to one or two short hyphae of one or two cells, although no secondary germ tubes developed (Fig. 1).

Erysiphe graminis on *Polypodium aurcum* and *Polypodium sp.* (*allied*). Infection proceeded normally to the papilla stage where it was stopped as on *Impatiens*. The cell wall round the point of penetration showed in unstained strips a bright yellow-brown halo which might be 20 μ wide. The haloes corresponded with colourless patches in strips treated with iodine and sulphuric acid and they developed from small yellowish circles like the cotton-blue haloes. A remarkable thing was noticed in this experiment, which was performed twice on each host at an interval of a week, each time with several leaves and a large number of spores. The penetration process always arose from a small tertiary germ tube: the primary germ tube lay along the leaf without apparently the least attempt at penetration (Fig. 1). The arrangement was so peculiar as to suggest that the host had had some effect on the germ tubes, and one could hardly suspect every conidium of the same irregularity.

Erysiphe graminis on *Adiantum reniforme*. Conidia would not germinate on old leaves, although these were placed on damp filter-paper in Petri dishes. On young leaves germination proceeded as readily as on *Polypodium aureum* up to the papilla stage, where it was stopped. The papilla was abnormally large, like a hemispherical pad; it was pierced nearly to the apex by a stout stylar process which, on falling out, left a relatively large conical hole. The surrounding

cell wall was discoloured bright rusty yellow as with *P. aureum*, but the altered area was smaller and the penetration process arose from the primary germ tube. Microtome sections of these papillae would be instructive in showing the exact shape of the penetration process.

Evidently the cellulose layer of the epidermal cells of these fern leaves differs chemically from that of the flowering plants with which I experimented, for such yellow discoloration was never observed with the latter. Yellowing, or browning, is a common feature of fern tissues, and the cytase from the penetration process may give some clue to its nature.

Sphaerotheca pannosa on *Impatiens Sultani*. Infection to the papilla stage with alteration of the cellulose layer took place exactly as with *Erysiphe graminis*.

Sphaerotheca pannosa on *Polypodium* spp. and *Adiantum reniforme*. Infection to the papilla stage with rusty yellow discoloration of the cellulose layer took place exactly as with *Erysiphe graminis*. All infections arose from the primary germ tube.

OBSERVATIONS WITH OTHER MILDEWS

Oidium euonymi-japonici. Conidia from *Euonymus japonicus*, germinated on the leaves of the host, produced a short, lobed, primary germ tube from which the first haustorium developed with the formation of a papilla and alteration of the cellulose layer as with *Erysiphe graminis* on a normal host. On *Impatiens Sultani* and *Adiantum reniforme* the conidia behaved exactly like those of *Erysiphe graminis* on these hosts.

Erysiphe cichoracearum. Conidia were taken from *Myosotis collina* and *Anchusa* sp. They infected the leaves of the host species exactly as in the case of *Erysiphe graminis* on a normal host. On *Impatiens Sultani*, *Taraxacum officinale*, *Convolvulus arvensis* and the three species of fern the conidia behaved like those of *Erysiphe graminis* on *Impatiens Sultani* and the ferns except that, after one penetration process had arisen from the germ tube and had been stopped at the papilla stage, a second and sometimes a third would arise from some point nearer the conidium, to be stopped at the same stage; in strips stained with iodine and sulphuric acid 2-3 colourless intersecting circles with a papilla at the centre of each, would be seen beneath the germ tubes. This is the only mildew in which I observed multiperforation: it is clearly connected with frustration because it did not occur on *Myosotis collina* or *Anchusa*. Conidia from *Myosotis collina* were also placed on leaves of *Veronica beccabunga*: after 48 hours

some primary germ tubes showed no sign of penetration, others had formed an abortive haustorium and yet others had developed normally to give rise to a full-sized haustorium and a secondary germ tube of 1-2 cells. It appears therefore that some conidia of this strain from *Myosotis collina* can parasitise *Veronica beccabunga*. As already noted, biologic specialisation in this species of mildew is particularly complicated; it would repay a detailed study.

The conical hole bored into the papilla by the stylar process was often of striking size on the leaves of *Impatiens* and *Taraxacum*. *Erysiphe graminis* also produced a large hole on *Impatiens*. The texture of the cellulose layer may differ in widely different plants.

CONCLUSIONS CONCERNING THE INOCULATION OF INAPPROPRIATE HOSTS

Given the right conditions for germination, mildew conidia will attempt to infect any plant on which they alight. If it can pierce the cuticle of an inappropriate host the penetration process is then stopped at the papilla stage and probably killed by toxic substances in the host cell. Whether the cellulose layer can act as a barrier is uncertain. The cytase, diffusing from the penetration process, may not be able to act on all kinds of cellulose, so that it would be interesting in this respect to germinate conidia on the leaves of horse-tails, lycopods, bryophytes and gymnosperms. Brown(3, 4) observed that the extract of the germ tubes of *Botrytis cinerea*, which contained a pectinase, had no effect on the species of bryophyte, fern and *Spirogyra* which he tried, though highly active in macerating phanerogamic tissue like that of potato tuber, leaves and petals. The cytase from the penetration process appears to be the same in all six species of mildew, as it produces the same effects. Chona(6) and Menon(14) have recently shown that the macerating enzyme, pectinase, is essentially the same in a variety of parasites.

That the germ tube should begin to penetrate on such a variety of unrelated hosts tends to prove that the stimulus to penetration is not provided by a chemical substance emanating from the host, but that it is merely a thigmotropic response. That a papilla should be formed in such a variety of hosts tends to prove that it is an effect of the penetration process on the cellulose layer and not a particular reaction of the host cell against the attack of mildews: the phenomenon of intersecting circles caused by multiperforation in *Erysiphe cichoracearum* also supports this contention.

NOTES ON THE GERMINATION OF MILDEW CONIDIA

It is known that the conidia of mildews do not germinate properly in water (10, 15, 16, 28). The conidia of the six species with which I experimented produced, when immersed in water, a short germ tube or commonly none at all: the vacuolate structure of the cytoplasm gradually collapsed into a granular homogeneous state, and after 24 hours, at *ca.* 20° C., the conidia were dead. Immersion of 1–3 hours was sufficient to destroy their power of germination. Spore emulsions are therefore useless for inoculation, and this peculiarity may explain Woodward's difficulty in obtaining germination (28).

On dry cover-slips in a saturated atmosphere, the conidia of all six species germinated normally. The apex of the germ tube was often capitate or lobed, as Neger (16) found, as if forming an appressorium on the cover-slip. One or two secondary germ tubes developed, but they remained short.

On the surface of water, however, the conidia germinated readily. They were dusted on to a drop in a watch-glass which was placed on water in a Petri dish. After 24 hours at *ca.* 20° C., in all six species, a single, unbranched, 1–2-septate germ tube, 100–200 μ long, had grown from each conidium vertically into the air. How the germ tube was balanced other than through sheer dexterity in apical growth it is impossible to say: isolated conidia produced such germ tubes and a tap on the watch-glass rolled them over. Abortive tertiary germ tubes had grown into the water in many cases and these might have assisted in the balancing by pressing against the underside of the surface film. As I looked at the forests of germ tubes, I saw that bubbles formed and burst round the conidia as if the water were beginning to boil. The bubbles must have been the carbon dioxide of respiration escaping from the water.

These simple experiments may be of interest in the physiology of mildews. For a fungus, growth of the germ tube into the air is clearly abnormal. The bubbling suggests that it is growth away from a high concentration of carbon dioxide to a high concentration of oxygen, and the relatively rapid death of the conidia when immersed in water suggests that they are killed from lack of oxygen rather than through any action of the water itself. One is led to postulate that mildews require a low carbon-dioxide tension and a high oxygen tension in their hyphae, wherefore they cannot normally enter stomata or become endoparasites, but for the most part are compelled to lead an ectoparasitic existence. Unlike most fungi, whose spores are packed

with dense cytoplasm and which absorb water and swell up on germination, mildews carry their water of germination in the highly vacuolate cytoplasm of the conidium in place of food reserves, and on germination they develop a haustorium at the first opportunity. That mildews will thrive in a hot, dry summer in England, when other parasitic fungi suffer through drought, is undoubtedly connected with these two facts: the ability of the conidia to germinate on a dry surface, and their inability to withstand prolonged wetting; rain must kill many spores if not the hyphae themselves. And so, too, with *Oidium heveae* in the eastern tropics, which in monsoon countries with a dry season may become a pest in rubber estates, yet rarely does so in regions of evergreen rain forest such as Malaya, Sumatra and the west of Java. The respiratory requirements of mildews in the vegetative state seem to be the antithesis of those of yeasts; in the ascigerous stage these requirements must be changed, for the hyphae combine to form an ill-aerated tissue.

Conidia which appear to be fresh may vary greatly, for no obvious reason, in their power of germination. I noticed this with rose and apple mildew. Conidia from vigorous young patches of mycelium would give perhaps 1 per cent. germination; under similar conditions I found 10–20 per cent. to be the average and 30–40 per cent. the maximum. With *Erysiphe graminis* I frequently obtained 100 per cent. germination, and this species was accordingly used in preference to the others. These irregularities have been noted by other investigators (10, 16). The life of mildew conidia seems to range from 2 to 3 days to a week, and a sample from an old patch of mycelium will always give a low percentage germination. The conidia germinate as soon as they are detached and they seem unable to withstand desiccation; long-distance infection by conidia is thus improbable.

SUMMARY

Experiments were made with *Erysiphe graminis*, *Podosphaera leucotricha* and *Sphaerotheca pannosa* to determine at what stage of infection resistant varieties of the host plant checked the attack of mildew. Norka and Persian Black were taken as resistant varieties of wheat, Worcester Pearmain of apple, and Gloire de Dijon and American Pillar of rose. Particular attention was given to the exact method of penetration of the host cell by the haustorial process.

The conidia germinated characteristically with a short, unicellular, primary germ tube from the tip of which the first haustorium

developed. On completion of the haustorium the apex of the primary germ tube resumed its growth and 1-3 secondary germ tubes grew out from the spore directly into hyphae. Abortive, simple or lobed, tertiary germ tubes might also arise.

It was concluded that the stimulus to penetration was thigmotropic, that the cuticle was pierced mechanically, that the cellulose layer of the host cell was swollen by a cytase diffusing from the penetration process, and that the infection papilla was formed by the intense local action of the cytase and the thrust of the process. The papilla and surrounding part of the cellulose layer were so altered by the cytase that they no longer responded to microchemical tests for cellulose, but the nature of the altered matrix was not determined.

On resistant varieties infection was generally stopped at the papilla stage without penetration of the host cytoplasm. Persian Black wheat was completely resistant. Norka wheat, Worcester Pearmain apple and Gloire de Dijon rose were imperfectly resistant in that subinfections with scant mycelium and rudimentary haustoria might develop on them and occasionally a normal mycelium with conidia.

On cross-inoculation with the physiologic forms of *Erysiphe graminis* from wheat, barley and *Agropyron repens*, the conidia also germinated and initiated penetration to the papilla stage on the wrong hosts; the process of penetration was then stopped and no haustoria were formed. The same results were obtained on a variety of inappropriate hosts, e.g. *Impatiens*, *Cobaea*, *Polypodium* and *Adiantum*, provided that the cuticle was not too thick. On *Polypodium* and *Adiantum* the penetration process turned the cellulose layer yellow-brown.

A few experiments with *Oidium cuonymi-japonici* and *Erysiphe cichoracearum* corroborated these results, which seem of general application to mildews.

The problem of resistance to mildews has been discussed. It is considered that resistance is primarily caused by toxins in the host cell, but that environmental and structural factors may also be operative, at least in cases of subinfection.

A short period (1-3 hours) of immersion in water was found to be sufficient to kill the conidia. When germinated on a water surface each conidium sent a long germ tube into the air. It is surmised that mildews required a high oxygen tension and a low carbon-dioxide tension in their cytoplasm.

These investigations, which were undertaken at the Cambridge Botany School during the year 1928, it has been impossible through change of circumstances to complete. But I wish to express my thanks to the Department of Scientific and Industrial Research for a grant in aid of the research, and especially to Mr F. T. Brooks, F.R.S., to whom I owe not only the suggestion of the problem but guidance and supervision, without which the results now presented could not have been obtained. It was Prof. W. Brown's invigorating address on the Mechanism of Disease Resistance in Plants to the British Mycological Society at Newcastle in September, 1933, which has prompted publication of my observations.

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NOTE ON THE STRUCTURE OF THE EPICOTYL IN *JUGLANS NIGRA*

By A. J. DAVEY

(With 2 figures in the text)

THE following account of seedling structure in *Juglans nigra* deals with the anatomy of the epicotyl and its early leaves. The work forms part of a study of the seedlings of the Juglandaceae which has arisen out of a previous survey of the seedling anatomy of the "Amentiferae" suggested by Dr E. N. Thomas.

It has been shown that the seedlings of *Juglans* species are exceptional on account of the part played in them by plumular traces in relation to root poles. In *Juglans nigra* the traces of the first two plumular leaves enter the axis as "double bundles" which constitute two poles of the tetrarch root independently of the cotyledon traces. The structure and behaviour of these two plumular traces is exactly similar to that which has been described by so many authors as characteristic of cotyledon traces. A similar connection of root poles with plumular traces has been recorded by Compton for two members of the Leguminosae, *Pithecolobium unguis-cati* and *Caesalpinia sepiaria*, and by the present author in *Castanea sativa*, but apart from these instances the feature seems to be rare or unnoted.

It has been found, as described in detail below, that in *Juglans nigra* doubleness of leaf traces is not confined to the first two plumular leaves, nor is it necessarily concerned in the formation of root poles. A double bundle, usually showing isolated centrally placed protoxylem elements, is present in the nodal region of at least six successive plumular leaves. The number of leaves varies slightly from one seedling to another, and recognition of the "double bundle" depends upon the examination of sufficiently early stages in development. The earliest xylem elements of the lower leaves may be already disappearing before the plumule has emerged from the seed.

Double plumular traces unconnected with root poles have been described by Thomas for the first pair of plumular leaves in *Cheiranthus maritimus* and in *Draba Aizoon* and by Holden and Bexon as occurring similarly in certain seedlings of *Cheiranthus cheiri*. *Juglans nigra* differs markedly from the above-mentioned species in respect of the greater number of leaves which possess the feature in question and the extent to which it persists.

Work on the species of *Juglans* has been much hindered by the difficulty of obtaining viable seed, especially of *J. nigra*. Only a very few of the nuts obtained in different years from various sources have germinated, and the time occupied in germination may be as long as two years. The facts described were first observed in a relatively old seedling in 1918, but between that time and the summer of 1924 all attempts to grow more material failed.

My thanks are due to Prof. Jeffery for seeds from the Arnold Arboretum, and to Messrs J. F. Jones (nut tree specialists, Lancaster, Penna) for seeds of *Juglans* and *Carya* species.

Among the species of *Juglans*, two types of plumule occur:

(a) That shown by *J. regia* and *J. cinerea* in which the first two leaves are compound foliage leaves approximating to the adult type of leaf. No scales or transitional forms of leaf are present, and there is no obvious double structure in the plumular leaf traces. This type of plumule is associated with the production of accessory buds in the cotyledon axils. In *J. regia* a vertical series of such buds arising in basipetal succession in the axil of each cotyledon is present on the embryonic axis in the seed.

(b) That shown by *J. nigra*, *J. Hindsii* and *J. sieboldiana*, in which the plumular axis bears a series of small-scale leaves. The first six or so of these are simple scales, but the succeeding ones are compound and show gradual transition towards the adult type of compound pinnate foliage leaf. It is in the scale leaves that doubleness of the median leaf-trace is clearly shown.

EPICOTYL STRUCTURE IN *JUGLANS NIGRA*

The chief interest lies in the structure of the vascular strands of the early leaves at an age when the plumule has lengthened little, and is still enclosed within the seed (Fig. 1). At such a stage, as many as six well-developed scale leaves may be seen below the terminal bud. The radicle has grown to a length of between 1 and 2 in., and the region of the cotyledonary node has increased considerably in girth as compared with younger stages. There is extension of the perimeter of the vascular ring together with much cambial activity. These developments cause alteration in the relative positions of root poles and leaf strands from those obtaining in younger seedlings. The changes are effected chiefly by the interpolation of new vascular elements developed from the cambium and by the disorganisation and disappearance of the earliest formed xylem elements.

The following details of structure are taken from the seedling

figured, and are typical of others examined. The plumule showed six scale leaves visible below the terminal bud, but sections of the apex revealed that in all twelve leaves were differentiated.

Numbering upwards from the cotyledons, the first five leaves are simple; the sixth and seventh are compound scales, each with a terminal leaflet and one pair of lateral pinnae; the eighth leaf bears two pairs of pinnae, while the succeeding leaves are present as very small rudiments. Axillary bud rudiments are present in the leaf axils.

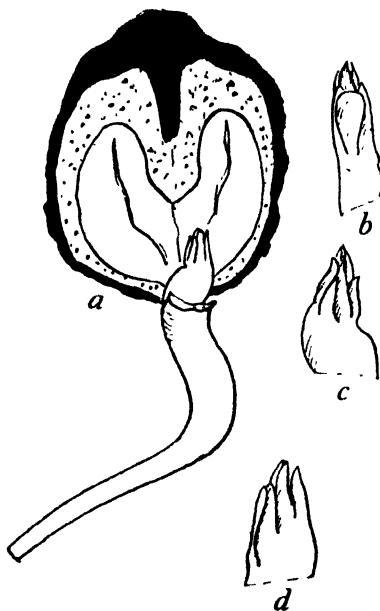


Fig 1. *a*, seedling of *Juglans nigra* with one cotyledon removed (natural size)
b, *c* and *d*, enlarged views of plumule showing scale leaves.

The anatomical features are described as they are met with in examining a series of sections descending from the apex. The primordia of leaves twelve, eleven and ten unite almost simultaneously with the flat apical region of the stem in which the vascular elements are not differentiated, but the position of desmogen strands is indicated. Leaf nine becomes attached at a slightly lower level, and is closely followed by leaves eight and seven.

The base of leaf eight and of successive older leaves contains an arc of vascular bundles varying in number but usually approximating to seven. As the node is approached, these bundles become aggregated into three groups: a medium group of three strands, and two

lateral groups, each of which coalesces into a single strand. The median group and the pair of laterals constitute the leaf trace which enters the axial stele at three gaps, in the manner regarded by Sinnott as typical for the leaves of dicotyledons. The lateral strands enter the ring at a slightly higher level than the median group.

In leaf eight the triple stranded median group becomes resolved into a double bundle by the union of one of its lateral strands with the central one. In this form the median trace enters the axial ring. Below this level the traces of this leaf do not possess lignified elements, and they cannot be identified with certainty at lower levels in the stem.

Leaf seven. This leaf consists of a central portion and one pair of lateral pinnae. Each pinna contributes a single strand to the rachis which then contains an arc of seven bundles. Towards the base of the leaf these become grouped as described for leaf eight, so that three strands form a median group and the remainder fuse to form two lateral strands. Near the node, the median group is resolved into a "double bundle" in the manner described for leaf eight. The two phloem groups of the double strand are separate and divergent, but nearer the node cambial development connects them. There are indications of earlier central xylem elements, but this is not so clearly shown as in lower leaves. The leaf trace enters the axial ring by the usual trilacunar gap, the median trace at a slightly lower level than the laterals. The double grouping of the phloem of the median leaf trace is recognisable in the axis until after the insertion of the next two lower leaves. Below this the leaf trace cannot be certainly identified.

Leaf six. This consists of a central slightly expanded leaflet with one pair of rudimentary lateral pinnae. After union of these pinnae with the main rachis, the latter shows an arc of about seven bundles, which become compacted, towards the base of the leaf, into three groups. Of these, the median group consists of three strands in each of which the xylem elements are few; most of them have been derived from cambium. At the level at which the leaf base fuses with the axis the lateral groups each become fused into a single strand; they enter the axial ring where their identity is soon lost, since at this level they are without lignified elements. The median trace becomes more compact, and while two early phloem centres are recognisable, cambial activity unites the rest of the group into one strand. Slightly below this level the group separates into two distinct portions which become more widely separated by parenchyma as the axis is descended. At

the same time lignified elements increase through the activity of the cambium. This leaf trace can be identified by means of its xylem groups to a level below that of the insertion of the next three lower leaves. Below this, there is absence of cambial activity between the two halves of the double trace, so that a gap is left at which the trace of the first plumular leaf will enter.

Leaf five. This is a simple scale leaf with an arc of vascular bundles segregating towards its base into three groups as described for above.

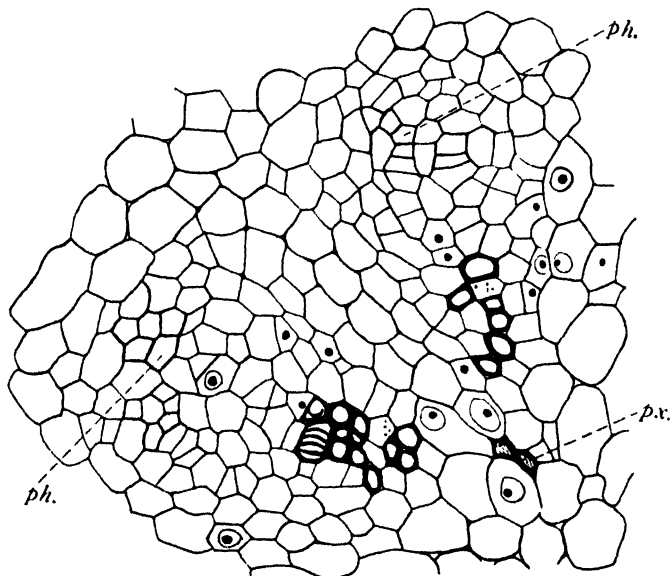


Fig. 2 Median leaf trace of the fifth plumular leaf of the seedling shown in Fig. 1. $\times 760$. $p.x.$ = protoxylem, $ph.$ = phloem.

Before the leaf has completely fused with the axis its median leaf trace becomes resolved into a double bundle by the union of its median strand with one of its laterals. Centrally placed protoxylem elements separated from the rest of the xylem by parenchyma are clearly differentiated in the nodal region of the leaf base, but die out after the leaf trace has entered the axial ring (Fig. 2). In descending the axis the halves of the double strand become more widely separated in correlation with the increasing girth of the vascular cylinder, and new groups of xylem and phloem elements formed by the cambium arise between them.

Leaf four. This is a simple scale with a vascular system similar to that of leaf five, except that only one lateral strand is present at

the node, the other having fused with the midrib at a higher level. Axillary bud meristem is present. Before joining the axial ring the median leaf trace is resolved into a double bundle. Isolated protoxylem is present in a central position, but shows signs of disorganisation. It cannot be recognised in the axis, where the halves of the double bundle become more widely separated.

Leaf three. This is a simple scale, whose base shows an arc of seven bundles, all of which converge towards the centre of the leaf and form, in the nodal region, a group of three strands corresponding to the midrib of higher leaves. One tiny lateral persists for a time but dies out before reaching the axis. An axillary bud is present which fuses with the axis almost simultaneously with the insertion of the leaf subtending it. The vascular system of the bud supplies to the axial ring of the epicotyl two small procambial strands. The median leaf trace, as in other leaves, consists of three strands, the xylem of which appears to be entirely secondary. As the node is approached, xylem elements already showing signs of disorganisation make their appearance internal to the central strand and separated from it by parenchyma. At a slightly lower level the leaf trace is resolved into two strands, while the centrally placed elements remain in a position midway between and internal to them. At this level there is considerable cambial development in the axis, and the vascular ring is continuous except for the single leaf gaps of this and the next lower leaf. As the leaf trace enters the ring its two components become more widely separated and the central protoxylem cannot be traced below the node.

Leaf two. This is a simple scale subtending an axillary bud. Its junction with the axis lies midway between the positions of insertion of leaves five and seven (Fig. 3), and is opposite that of the first leaf. These two leaf insertions lie in the plane passing between the two cotyledons (intercotyledonary plane) in which at a lower level lie two opposite poles of the tetrarch root. The vascular system of the leaf base consists of an arc of bundles which fuse together, forming a three-stranded midrib as the node is approached. This midrib is resolved into a double bundle whose two components diverge widely after entering the axial ring. Central xylem makes its appearance as an isolated element, and in seedlings of this age soon dies out. The gap between the halves of the double bundle widens considerably. In a seedling of this age cambial divisions extend from either side of the gap so as almost to bridge it, but (as shown previously) in earlier stages the cambium is absent.

Leaf one. Leaf one joins the axis at a slightly lower level than leaf two. The behaviour of its leaf trace resembles that of leaf two.

In the first plumular internode the amount of phloem present is increased by the formation of small groups, which are interpolated between the original traces. Further development of phloem from the cambium links the groups into continuous bands, gaps remaining opposite one another in the intercotyledonary and cotyledonary planes. Near the cotyledonary node metaxylem elements appear on either side of the intercotyledonary gaps. These elements increase in number as the axis is descended, and extend as tangential bands towards the middle of the gap which will be the position of the root pole.

At a slightly lower level a small group of xylem elements appears in the centre of the gap; as the axis is descended further additional elements are found external to this group which is thus gradually extended outwards. The last elements to appear are those of the early protoxylem. Meanwhile the stele becomes contracted and the tangential bands of xylem become united with the central group, thus forming a xylem pole of the root which is flanked by the downward continuation of the halves of the plumular trace.

Cotyledons. Each cotyledon petiole is broad and flattened and inserted on the stem by a broad base and possesses a number of vascular bundles arranged in two laterally placed arcs, a midrib being absent. As the axis is approached, fusion of strands reduces each lateral arc to a group of three strands, and each of these groups is then resolved into a double bundle exactly as has been described for the midrib of plumular leaves. Each cotyledon thus contributes two double bundles to the axial stele. They enter by gaps diagonally placed. Centrally placed protoxylem elements appear internal to their main xylem and appearances may suggest for a time that four root poles will be organised in the diagonal position, one from each double bundle (making with the plumular poles six in all). In fact this occasionally happens, and the root is then hexarch. Usually the double bundle becomes very compact as it enters the axial stele, and its xylem groups are deviated towards the cotyledonary plane where a small gap is present flanked on either side by strands from the cotyledonary buds. These soon die out and there is a gradual approach of the cotyledonary xylem groups towards the cotyledonary plane where they become related to the cotyledonary root poles.

In a seedling of this age the exact relationship of the earliest xylem elements of leaf and cotyledon traces to root poles cannot be

seen, for the following reasons. The parenchymatous gaps between the halves of a bundle, and between other bundles, increase in width. By this means the perimeter of the stele as a whole is extended. At the same time cambial divisions in the normal radial direction increase the thickness of the ring, and from cells so formed in the gaps new phloem and xylem elements arise, while the earlier protoxylem elements are stretched and strained by longitudinal growth of the living cells around them so that they finally disappear. (Cf. description of younger seedlings (Davey, 1916).)

SUMMARY OF THE BEHAVIOUR OF PLUMULAR LEAF TRACES

The first three leaves each supply one leaf trace to the stem. The fourth leaf supplies a median trace and one lateral strand, while all the succeeding leaves supply each a median trace and two laterals which enter the axial ring by a trilacunar gap in the manner found by Sinnott to be characteristic of the leaves of dicotyledons.

In the leaf base and at the node, the median leaf trace consists of two collateral strands separated by parenchyma. The earliest xylem elements are found in a small group internal to the double bundle and separated from it by parenchyma. This central xylem does not generally persist far below the node, but the halves of the double bundle remain distinct and become more widely separated as the axis is descended. In the axis it becomes difficult and almost impossible to identify the traces of the younger leaves after lignification dies out in them, because early and rapid cambial development makes the axial ring practically continuous except at leaf gaps.

The form and structure of successive leaves on the plumular axis is not identical, hence young stages seen in the higher leaves will not reproduce exactly the structures seen in lower leaves at corresponding ages. The structure described for the median leaf trace is more strongly marked and persists for a longer period in the lower leaves than in the higher ones. In proceeding upwards from lower to higher leaves the central protoxylem is less in evidence and persists for a shorter time; the two phloem centres are separated by a narrower ray of parenchyma and cambial activity bridges this more rapidly.

It is clear that in the earliest phase of its development at the node in the nodal region, the median leaf trace consists of a very few centrally placed xylem elements (often one only) flanked by two centres of phloem development, more or less widely separated by parenchyma. This primary structure is comparable with the alternate

¹ See Thomas, Compton, Chauveaud, etc., *loc. cit.*

arrangement of xylem and phloem found in the young hypocotyl of most seedlings and described in various terms by different authors. Cambial development internal to the phloem groups occurs at a very early stage so that most if not all of the xylem elements other than the central ones are secondary in origin.

GENERAL REMARKS

The presence in plumular leaves of double strands unrelated to root poles lends support to the suggestion put forward by Thomas that "doubleness" may be a primitive foliar arrangement. In this connection it is significant that only the median leaf traces of *Juglans nigra* are double.

Doubleness of the median leaf trace characterises those leaves which are laid down in the embryo and have their final form more or less completely realised while still within the seed. Such leaves occur in many large hypogeal seedlings in which, on germination, the epicotyl resumes growth within the seed under conditions similar to those in which it was first laid down in the embryo.

This is in strong contrast with what obtains in most epigeal seedlings where growth of plumular bud is retarded and takes place above ground external to the seed. For example, doubleness is not obviously present in the plumular leaf traces of *Juglans regia* in which species scale leaves are absent from the axis, and the first two foliage leaves carry out most of their development above ground after emergence from the seed.

It is possible that the occurrence and degree of persistence of "doubleness" in the strands of both cotyledons and foliage leaves may be determined by the extent to which both types of leaf are subjected to the special conditions which control growth and development of the embryo within the seed, and that therefore "doubleness" may not necessarily be of phylogenetic significance. On the other hand, if "doubleness" be regarded as a primitive feature, it may be that development within the seed is responsible for the factors which cause it to be retained in the early life of the seedling.

SUMMARY

In the seedlings of certain species of *Juglans* (notably *Juglans nigra*) the early plumular leaves possess a double leaf trace with isolated central protoxylem.

The traces of the first two leaves are directly connected with root

poles, but the traces of a number of the succeeding leaves have no direct connection with root poles, and their protoxylem does not penetrate into the root.

Such leaf traces seem to be characteristic of scale leaves of limited growth whose form is determined while still within the seed.

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THE UTILISATION OF ORGANIC ACIDS BY *ASPERGILLUS NIGER*

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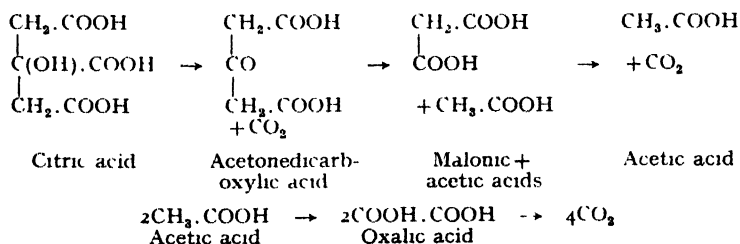
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INTRODUCTION

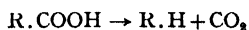
MANY mould fungi (species of *Aspergillus* and *Penicillium*, especially) produce citric and other organic acids when grown on sugar solutions. These acids accumulate to a maximum concentration, and, as starvation progresses, the acid finally disappears. The whole process closely resembles the accumulation of malic acid in succulent plants at the expense of sugar, and its eventual disappearance as starvation of the tissues progresses.

Some controversy still exists as to the significance and chemical mechanism of the formation of these acids, but a considerable number of workers agree that the disappearance of these acids, both in succulent plants and the mould fungi, is brought about by their oxidation resulting finally in conversion of the acids into CO_2 and water. One of us has already produced evidence that the disappearance of malic acid from the tissues of succulent plants is due to reduction rather than oxidation (1, 3), and it appeared desirable to reinvestigate the fate of acids disappearing from fungus cultures, and to compare as closely as possible the respiratory phenomena of these two groups of plants.

Since accounts of the acid metabolism of mould fungi and of succulent plants have appeared recently (2, 3, 4), it will suffice to point out that it is generally assumed that the disappearance of citric acid effected by these plants is due to the occurrence of the following reactions:



It will be noted that, provided that the intermediate products do not accumulate, six molecules of CO_2 should be evolved for each molecule of citric acid which disappears. Decrease in titratable acidity of living tissues is almost universally assumed to be due to the occurrence of reactions of the type



both in fungi and in the tissues of the higher plants. The conversion of citric to acetonedicarboxylic, or of oxalic acid to carbon dioxide are reactions of this type. If the disappearance of these acids from mould fungus cultures is brought about by these reactions, it follows that a decrease in titratable acidity of one equivalent must be associated with the evolution of at least one mol of carbon dioxide.

Our experimental results described below show that the disappearance of citric and other acids from cultures of *Aspergillus niger* is not accompanied by the production of carbon dioxide, and it follows that its disappearance must be due to other reactions than those which are generally accepted as valid at the present time.

EXPERIMENTAL METHODS AND MATERIAL

In all the work described in the present paper a single strain of *A. niger* was used¹; which had been isolated as a "single conidial-head" culture from a contaminated cotton boll.

The cultures used for experimental purposes were obtained by inoculating Czapek's solution made up with 5 per cent. glucose with a spore suspension obtained from the stock cultures grown on potato agar. These experimental cultures were grown and investigated in special squat-shaped hard glass flasks. The temperature at which the fungi were grown was in all cases 23°C . In order to maintain a low concentration of CO_2 in these culture flasks, a stream of CO_2 -free air was drawn through at the rate of about 3 litres per hour. The CO_2 in the issuing stream of air was absorbed in $2N$ NaOH contained in Pettenkofer tubes. Sterility of the inflowing air stream was effected by passing it through a long potash tower and also bubbling it through a flask containing concentrated caustic soda. The connections to these parts were sterilised, and the air stream also passed through tubes packed with cotton-wool and sterilised. The exit tubes from the culture flasks were also guarded with sterilised tubes packed with cotton-wool. The flasks were provided with tubes by which the culture solution could be run out completely from under the mat of

¹ Catalogued as M 1.

fungus mycelium. Samples for analysis were taken at regular intervals also by means of these tubes.

Control experiments showed that the various precautions for prevention of contamination were entirely adequate.

The methods used resembled in certain features those adopted by Kosinski (9). The cultures were grown on the Czapek glucose solution for various periods of time, and the culture solution was then washed out and replaced by sterile distilled water as in Kosinski's work. After some given period of starvation, the distilled water was run off and was replaced by solutions of nutrient salts and glucose, or by solutions of various organic acids.

The effects of these treatments on the rate of carbon dioxide output and the change in compositions of the solutions were investigated.

Of the many experiments carried out two types will be discussed in this paper. Firstly, those conducted in order to find out the effects of time (and therefore age of the fungus mat) on the concentrations of sugar and other substances in the solution, and on the rate of CO_2 output when the solution on which the spores started to grow remained unchanged throughout the whole course of the experiment. Secondly, experiments in which this original solution was run out and replaced with water, and in which the water was eventually run out and replaced with solutions of certain organic acids.

EXPERIMENTAL RESULTS

Group I

In experiments of this group a spore suspension was added to 500 ml. of Czapek glucose containing 5.0 gm. glucose, 0.3 gm. NaNO_3 , 0.1 gm. MgSO_4 , 0.2 gm. KH_2PO_4 , and 0.002 gm. FeSO_4 per 100 ml. of solution. The culture flask used as described had a diameter of 18 cm., and the maximum area of the mat of fungus was therefore 254 sq. cm.

The rates of respiration and the shapes of the respiration-time curves were found to be very variable. This variability is to a large extent determined by the number of spores sown in the culture flask. The nature of the observed effects are clearly illustrated by two cultures representing the two extreme types of respiration time curve.

These cultures, A 10 and B 10, were sown at the same time on the standard solution described; A 10 was inoculated with 5 ml. of spore suspension and B 10 with one drop of the same spore suspension. Both

cultures were grown at 23° C. and were aerated as described from the time of sowing of the spores. The respiration-time curves of the two cultures are given in Fig. 1. It will be noted that the rate of CO₂ output is expressed in mg. per hour per culture.

A high maximum rate of CO₂ output is attained by A 10 at about the 5th day after sowing; during these first 5 days the mycelium rapidly spread over the surface of the culture solution and formed a very thick mat on which conidiophores began to appear about the 5th day, and on the 7th day the whole surface was dark brown with

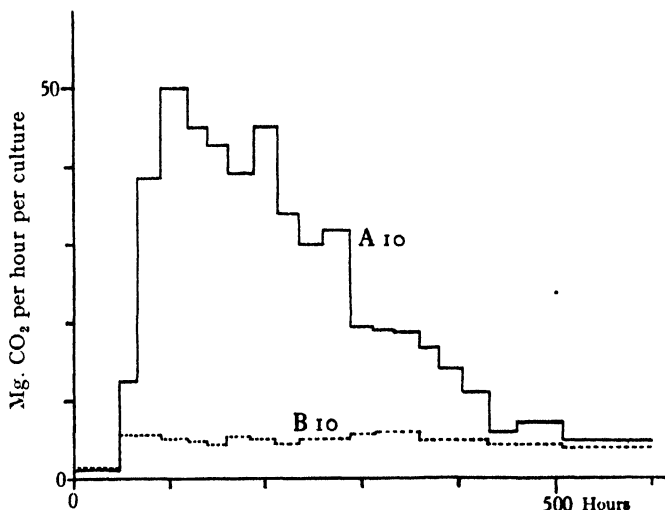


Fig. 1. Respiration-time curves of cultures A 10 and B 10.

ripe conidia. From this time on, a slow continuous decrease in rate of CO₂ output occurred, which is apparently correlated with the simultaneous decrease in sugar concentration (see Fig. 2). Judging from the appearance of the culture, it would appear that the initial rise in rate of CO₂ output is due to the growth of the mat of mycelium which appears to attain its maximum size about the 5th day.

The weight (and to some extent the composition) of the mycelium is obtained at intervals from the various analyses of samples of the culture solution made from time to time. In this experiment the following data were obtained: (a) titratable acidity, (b) total oxalate by Bau's method, (c) glucose by Benedict's method and polarimetrically, (d) total carbon in solution by Birkinshaw and Raistrick's (6) wet combustion method, (e) nitrate in solution by the

Devarda alloy method, and (f) CO_2 output by the Pettenkofer method as described. The total quantities are calculated from analyses carried out on small samples, usually 5 ml. or less.

At the start of the experiment there were 25 gm. of glucose, i.e. 10 gm. of carbon, present. The weight of carbon in the mycelium at any time is consequently $10 - (f + d + D)$, where f is the weight of CO_2 carbon evolved, d the weight of carbon in the solution, and D the weight of carbon removed from the solution in the various samples taken for analysis. The nitrogen content of the mycelium is obtained similarly.

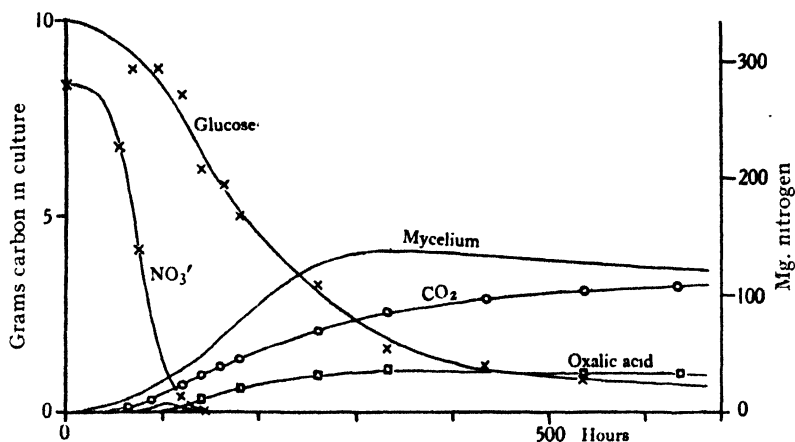


Fig. 2 Progress curves for culture A10 showing quantities of carbon left as glucose - x -, converted to CO_2 - o -, converted to oxalic acid - \square -, converted to citric acid - unlabeled curve with maximum at 100 hours, converted into mycelium - —. The quantity of nitrate-N left in the culture solution is given by the curve - x -, the ordinates for which are at the right side.

Most of these data are recorded in Fig. 2; age of the culture is plotted on the horizontal axis and the quantity of nitrogen (as nitrate) in the solution and the quantities of carbon present in various forms in solution and carbon in the mycelium are given as ordinates. The quantities of carbon in the forms of glucose, citric and oxalic acids, total CO_2 , and fungus mycelium are recorded. As the record indicates, the total carbon in solution is almost exactly equal to the sum of glucose-C + organic acid-C. Only very small traces of other carbon compounds can therefore be present in the culture fluid.

It will be noted that nitrate is rapidly absorbed by the growing mycelium, so that after 6 days all the nitrate has disappeared from

the culture solution and, clearly, no further increase in nitrogen content of the mycelium is possible. Correlated with this the mycelium did not appear to grow larger, but, as the data of Fig. 2 show, it must actually have increased largely in weight since, during the 10 days following attainment of maximum nitrogen content, the carbon content of the mycelium increased from 1.6 to 4.1 gm. It seems possible that the greater part of this increase is due to the laying down of reserves of polysaccharide, since microscopic examination shows that at this stage of development the hyphae become more and more densely filled with glycogen granules. These granules are stained reddish orange with iodine and are described here consequently as glycogen; some investigators (Schmidt (12)) have described the presence of a blue-staining polysaccharide ("starch") in *A. niger*, but no such substance was detected in our strain.

These large glycogen reserves are capable of maintaining the low starvation rate of CO_2 production for very long periods of time.

Among other things these results indicate clearly that the dry weight of the mycelium is not a suitable unit to which to refer the rates of physiological processes such as respiration. It seems improbable that the "active surfaces" of the protoplasm increase in quantity to any considerable extent after the maximum nitrogen content has been attained, though the dry weight increases 3-5 times after this point. We are consequently referring respiration rates not to unit dry weight of mycelium but to a standard mat of definite area (254 cm.²) and definite nitrogen content (280 mg.).

The culture B 10, as Fig. 1 shows, behaved quite differently. The mycelium formed a thin skin of hyphae all over the surface of the culture solution. This thin mycelium used up essential nutrient materials slowly and maintained a low constant respiration rate for many weeks. More recent work shows that these thin mats are developed both when spores from staling cultures are used, and when the spore suspension used for inoculation is heated unduly in the case of spore suspensions obtained from fresh cultures. The temperature of the sterile pipette used for inoculation is of some importance consequently.

The results are not dealt with in the present paper, but it is found that the "thick type" of mat (e.g. A 10) produces only very small traces of citric or gluconic acid. Note, for example, the small curve, with its maximum at the 100th hour, in Fig. 2, which indicates the quantity of citric acid produced by A 10. This type of mycelial mat produces large amounts of oxalic acid, as the same figure shows. The

"thin type" of mat, on the other hand, produces considerable quantities of gluconic and citric acids, and oxalic acid only accumulates when starvation conditions arise.

Many cultures are of course intermediate in type both as regards their respiratory activity and biochemical behaviour. One must emphasise that these striking differences in biochemical behaviour are obtained with cultures of the same strain. All the cultures referred to in the following pages are of the active "thick type".

Group II

Culture B6. This was grown under the standard conditions which have been described. The record of rate of CO₂ output from the time of sowing of the spores is given in Fig. 3. The culture solution was

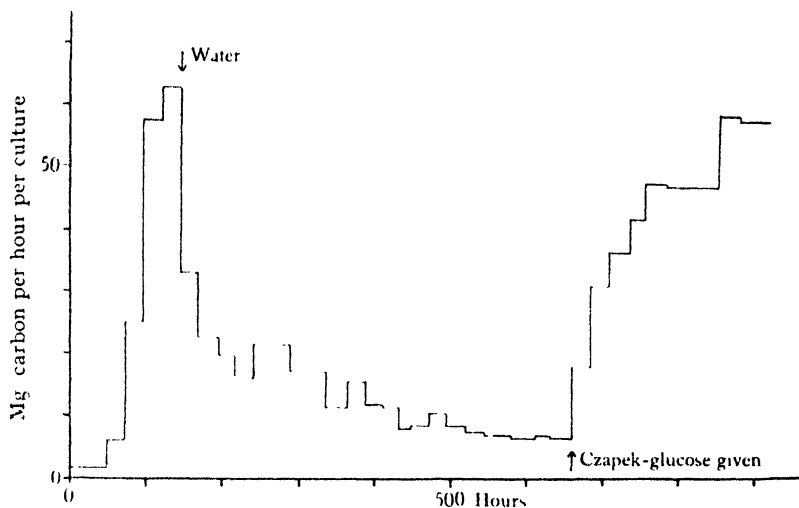


Fig. 3 Respiration-time curve of culture B6 showing the effects of starvation and resupply of the original culture solution

run out on the 6th day as noted by the first arrow on the graphical record. The mat of mycelium was washed with distilled water added under sterile conditions, and was resupplied with 500 ml. of sterile distilled water. This treatment causes the respiration rate to fall off roughly logarithmically to a low starvation rate which is maintained for many days.

At the time indicated by the second arrow this water was run out and replaced by 500 ml. of the standard culture solution (5 per cent. glucose Czapek). The respiration rate rises steeply and soon

attains a value roughly equal to that obtaining before the period of starvation commenced. Long periods of starvation such as this apparently have no permanently injurious effects on the fungus. Microscopic observation also shows that they do not entirely deplete the mycelium of glycogen reserves. It seems reasonable to infer that the slow "starvation respiration" is derived from breakdown of sugars set free by glycogen hydrolysis, and that the presence of high external sugar supplies enables the rapid "floating respiration" rate

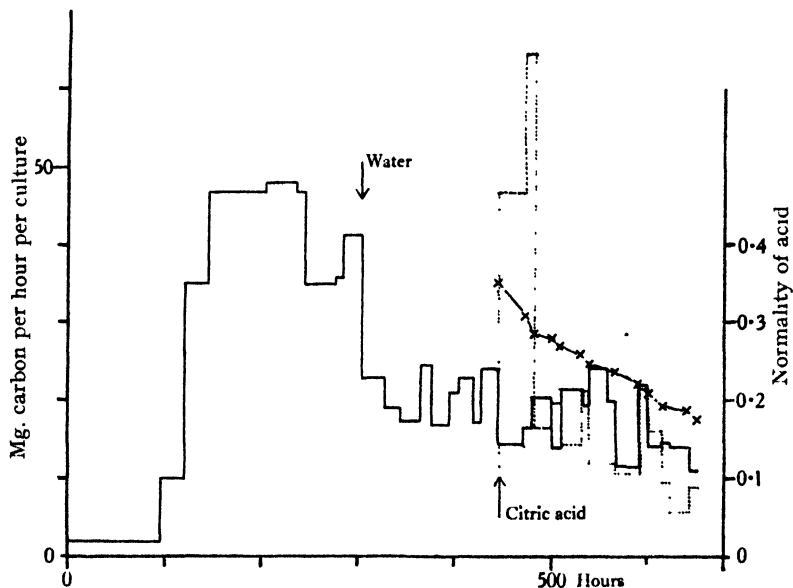


Fig. 4. Respiration-time curve of culture B1 (—) showing effects of starvation and resupply of citric acid. Rate of disappearance of citric acid shown by stepped dotted record in mg. C per hour. Actual concentration of acid shown by record - x -, the ordinates for which are on the right side

to be maintained. These results are in general agreement with the conclusions of Kosinski (9).

Culture B1. This was grown under standard conditions described before. After 300 hours (first arrow in Fig. 4) the culture solution was run out, the mycelial mat washed twice and 500 ml. of distilled water replaced, all these operations being carried out under sterile conditions. The treatment and the results are essentially similar to those already described for culture B6. The rate of respiration is recorded for this and all subsequent experiments in terms of mg. of carbon evolved in the form of CO_2 instead of mg. of carbon dioxide,

in order to facilitate comparison of the rate of disappearance of carbohydrate, citric acid, etc., with rate of output of carbon dioxide.

After 148 hours of starvation the water was run out from the culture and 500 ml. of 0.3*N* citric acid was supplied (second arrow in Fig. 4). It will be noted that the CO₂ output is not influenced by this treatment, and the rate of output remains at the same level as during the starvation phase. It seems probable, therefore, that the citric acid is not converted even partially into CO₂. This is remarkable, for titration shows that the acid disappears rapidly. The change in concentration with time is given by the curve and the points -x- in Fig. 4 and the actual rates of loss of acid are given in terms of mg. carbon lost per hour per culture by the stepped dotted record.

The rate of disappearance of citric acid was obtained by the titration of aliquot samples of the culture fluid. These results agreed well with determinations of citric acid by conversion into penta-bromacetone. Qualitative tests of samples of the culture solution resulted in failure to detect even traces of either acetone-dicarboxylic or oxalic acids.

At the beginning of the period of supply of citric acid, the rate of loss of citric acid-C was about three times as great as the CO₂-C output. The rate of loss of acid eventually decreases to zero when all the acid has been used up. During this process the CO₂ output remains constant, and the whole of this output can be accounted for as being due to the reaction sequence:



The citric acid which disappeared could not be recovered by extraction of the mycelium with water or ether; its disappearance is therefore not due to accumulation inside the mycelium.

The only available bases which might neutralise the acid are ammonia or ammonia derivatives which might conceivably arise from protein. The original culture solution only contained 18 mg. atoms of nitrogen, and clearly not all of this could be available. 64.4 mg.-equiv. of citric acid disappeared during the first 27 hours after supply of citric acid. The quantity of acid disappearing is therefore greatly in excess of any base which might be produced to neutralise it by converting it to either a salt or amide.

Cultures A 1, Bb 2, and C 8. These were carried out in the same way as B 1. After a period of starvation the cultures were supplied with acid, A 1 and Bb 2 with 0.3*N* citric acid and C 8 with 0.14*N* citric acid. In both cases, as the records in Figs. 5 and 7 show, the initial

rate of acid-C loss greatly exceeds that of CO_2 -C loss. The dosage with citric acid appears to have no effect on the rate of carbon dioxide

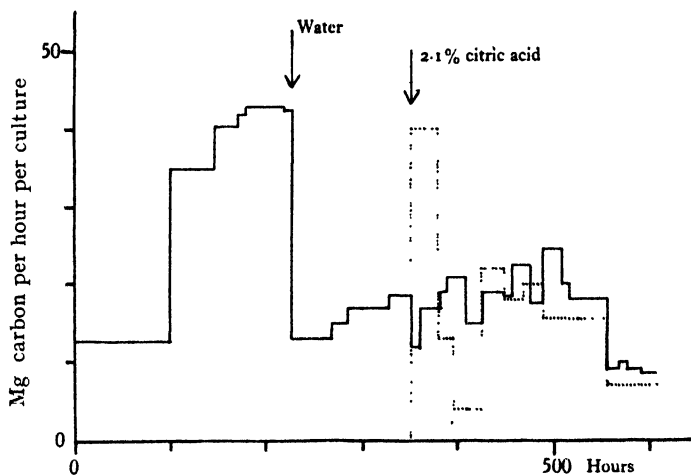


Fig. 5. Respiration-time and acid loss-time curves for culture A1

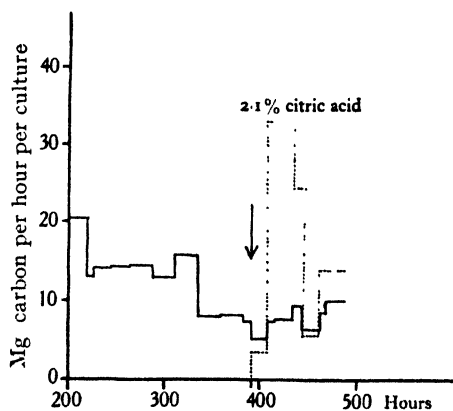


Fig. 6. Respiration-time and acid loss-time curves for culture Bb2.

output which remains at the starvation level. It will be noted that the first part, about 200 hours, of the record of Bb2 is omitted; this part of the record is closely similar to the first 200 hours of the record of B1 (fig. 4).

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Culture Bb1. In this case the fungus mat was grown for the first 200 hours on Czapek solution made up with 10 per cent. sucrose instead of glucose; this solution was then run out and the mat washed with sterile water in the usual way. But it was supplied with a 0.014*N* solution of citric acid instead of water as in the previously described experiments. The effect was the same as when water was supplied; the CO₂ output fell rapidly to the starvation level. The record in Fig. 8 shows that the citric acid had all been used up in 70 hours. The rate of disappearance of acid is, as before, given by the dotted stepped record.

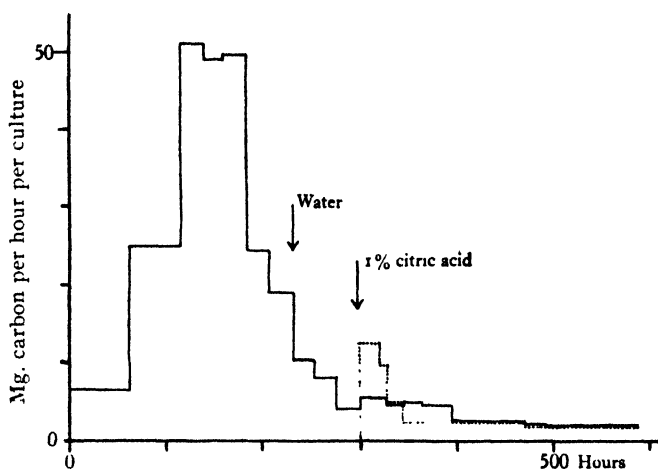


Fig. 7. Respiration-time and acid loss-time curves for culture C8

The liquid was run out and replaced by a second supply of citric acid solution of approximately the same strength. This also disappeared at about the same rate. The carbon dioxide output remains at the starvation level. After this second supply of citric acid had been used up the liquid was run out and approximately 0.01*N* oxalic acid was replaced (third arrow in Fig. 8); this also rapidly disappeared, and its disappearance was not associated with any rise in rate of carbon dioxide output above the starvation level. The rate of oxalic acid carbon loss roughly equals the starvation rate of CO₂-C output.

Cultures A9 and B9. These two were grown simultaneously in the ordinary way on 5 per cent. glucose Czapek solution. Both were of the active type and were closely similar to each other. The carbon

dioxide outputs of both are given by the stepped records in Fig. 9. The decrease in respiration rate from the maximum value appears to be due to "starvation", as the following data indicate. The glucose content of the solution of A9 had fallen to 0.92 per cent. at the 124th hour; the glucose contents of both cultures were 0.01 per cent. at the 196th hour and approximately 0.003 per cent. at the 242nd hour. At this point (first arrow in Fig. 9) the culture solutions were run out and replaced with sterile distilled water. 24 hours later (second arrow) the water was run out from A9 and was replaced with a 0.28*N* solution of glycollic acid, B9 was left as a control supplied only with water.

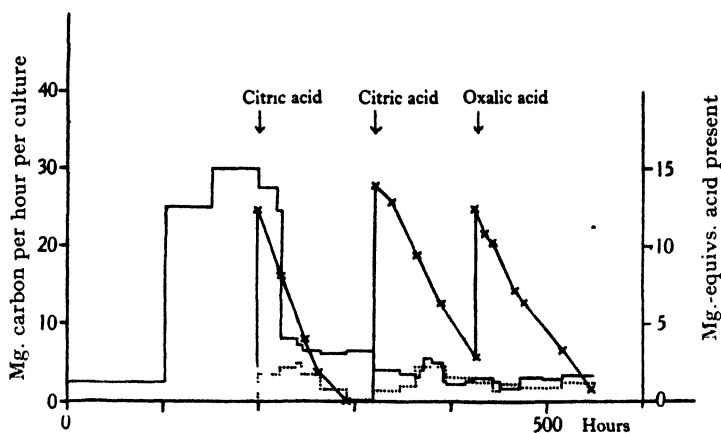


Fig. 8. Respiration-time and acid loss-time curves for culture Bb1. Actual quantities of acid in the culture fluid are shown by the records -x-, the ordinates for which are on the right.

It will be noted that the courses of carbon dioxide output of the two cultures remain strictly parallel throughout the whole period including the final stage when A9 is supplied with glycollic acid and B9 with water. The glycollic acid disappears rapidly, the change of concentration is given by the curve -x-, and the rate of disappearance by the dotted stepped record. This rate is slightly higher than the starvation rate of CO₂ output.

Both cultures produced considerable amounts of oxalic acid during the phase when glucose supplies were plentiful. The total quantity produced by A9 was 0.810 gm. and by B9 0.0570 gm. All other cultures of the "active" type of this strain of *A. niger* also produced oxalic acid from glucose in considerable quantity. It is therefore

remarkable that not even traces of oxalic acid were detected in the cultures supplied with glycollic acid or in those supplied with citric acid.

It is not proposed to give details of many other experiments the results of which do not differ materially from those already described.

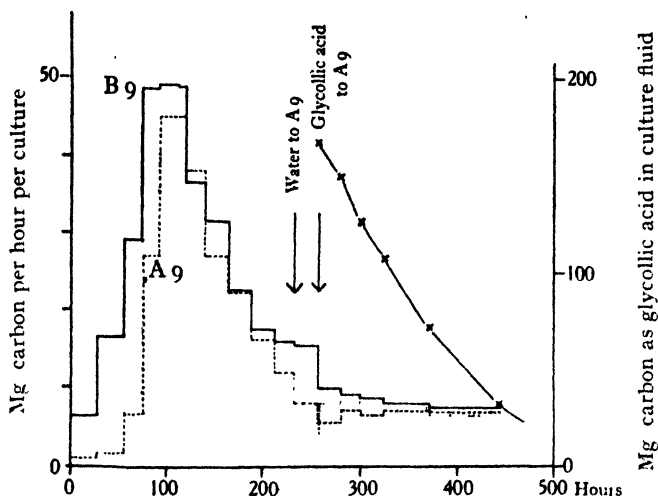
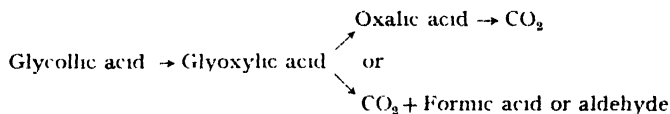


Fig. 9. Respiration-time curves of culture A9 ---- and B9 ——. Rate of disappearance of acid in carbon units given by stepped dotted record. Actual quantities of glycollic acid given by record -x- with ordinates on right.

DISCUSSION

(a) Special interest attaches to the fate of glycollic acid when supplied to cultures of *A. niger*. It is very generally assumed that the process involved in its disappearance is as follows (1, 8):



Raistrick and Clark (11), also Bernhauer and Scheuer (5), have shown that oxalic acid does not accumulate in certain *A. niger* cultures supplied with glycollic acid, even though similar cultures can accumulate it when supplied with such varied substrates as acetic, malic, or citric acids, or glucose. These facts taken alone do not establish the fact that the reactions mentioned above do not or cannot take place. If the velocity constant of formation of oxalic acid is less than

that of its decomposition, the upper sequence of reactions might take place without any marked accumulation of oxalic acid occurring.

The behaviour of our culture A9, however, establishes the non-occurrence of any of the above reactions, since rapid disappearance of glycollic acid is found to be associated with no extra production of *either* CO_2 *or* oxalic acid. Since the titratable acidity decreases it is also quite clear that accumulations of 4-C acids or citric acid do not take the place of oxalic acid. Oxidation of the glycollic acid therefore does not take place.

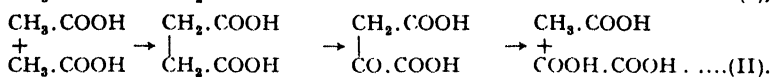
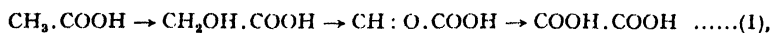
Its disappearance is almost certainly not due to accumulation of the acid or its anhydride glycolide in the mycelium, as repeated extraction failed to recover more than small traces of these substances from the mycelium. Conversion of the acid into salts or amide derivatives is ruled out by various considerations. The mycelium removed altogether approximately 60 mg.-equiv. of glycollic acid, and it contained at most 8.8 mg. atoms of nitrogen, this being the quantity supplied in the original culture solution.

The following causes of disappearance of the acid are therefore ruled out as impossible: (a) accumulation in mycelium, (b) conversion to anhydrides, (c) conversion to amide or salts, (d) oxidation, which would necessarily result in CO_2 or oxalic acid production. There remains only one simple alternative reaction which could cause the removal of the acid, namely its reduction to glycollic aldehyde. Glycollic aldehyde certainly could not be detected in the culture solution outside the fungus; this is not altogether surprising, since if it is formed it is almost certain that it will be formed at an active enzyme surface and will probably undergo polymerisation there.

Ordinary aqueous solutions of glycollic aldehyde are highly unstable and readily undergo polymerisation, the products being carbohydrates. It seems therefore highly probable to us that the disappearance of glycollic acid is due to reduction to the aldehyde and polymerisation of this to carbohydrate, possibly to glycogen. The first of these reactions is an endothermic, and presumably a coupled reaction. Direct positive evidence in favour of this point of view has so far not been obtained, but we submit that the negative evidence put forward possesses very great cogency in view partly of the simple constitution of glycollic acid and limited number of reactions in which large quantities of it could take part.

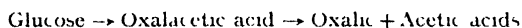
(b) The fact that it is quite definitely established that oxalic acid is not formed from glycollic acid emphasises again various difficulties in accounting for the mode of origin of oxalic acid. It has been

pointed out already by one of us(3) that these facts show that conversion of acetic acid to oxalic acid must proceed by the second of the series of reactions given below: rather than by the first which has hitherto been accepted as the most probable course:



The final reaction of series (I), the conversion of glyoxylic to oxalic acid, was shown by Raistrick and Clark not to take place, at least oxalic acid did not accumulate. The final reaction of series (II), the "acid hydrolysis" of oxalacetic acid, has not yet definitely been shown to occur in *Aspergillus*, though it is generally assumed to be the probable fate of part of any oxalacetic acid which may be produced by the fungus(4).

As shown in the experimental part of this paper, citric acid supplied to well-aerated cultures is not even partially converted to oxalic acid. Other exactly similar experiments not described here show that malic acid disappears rapidly from such cultures without being converted into either oxalic acid or carbon dioxide. Glucose, on the other hand, is partially converted to oxalic acid which accumulates at a considerable rate. Now since glyoxylic acid is not the immediate precursor of oxalic acid, oxalacetic acid probably is, and one can write schematically the sequence of reactions below as the probable mode of origin of the oxalic acid formed in cultures supplied with glucose:

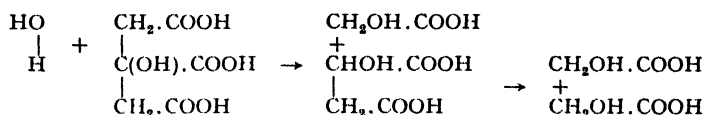


The fact that cultures supplied with malic or citric acids under exactly similar conditions produce no oxalic acid whatever very strongly suggests that the process of removal of malic and citric acids does not involve their conversion to oxalacetic acid. Hitherto it has been almost universally assumed that the first stage in the further conversion of malic acid in living organisms is its oxidation to oxalacetic acid (cf. (4), (13)).

(c) The experimental work described above shows that citric acid disappears rapidly from well-aerated cultures of *A. niger*. No carbon dioxide in excess of the starvation rate is produced, and neither oxalic nor acetonedicarboxylic acids are detectable. Under these conditions oxidation of citric acid according to the generally accepted scheme of Challenger, Subramaniam, and Walker(8) seems improb-

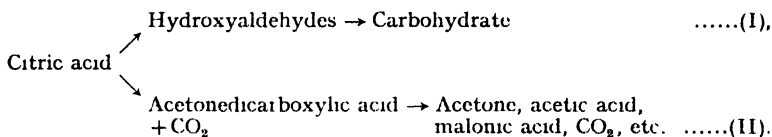
able. Conversion of the acid into salts, amides, or anhydrides also seems improbable for reasons which have been detailed (p. 219).

Relatively few remaining fates are possible for the disappearing citric acid. The actual loss of titratable acidity (as in the case of glycollic acid previously dealt with) is most simply explained as being due to reduction of the carboxyl to carbonyl groups. It does not seem very probable that the branched chain aldehyde of citric acid itself is formed. Straight chain hydroxyaldehydes might be formed if preliminary hydrolysis of the citric acid occurred as below:



Glycollic and malic or only glycollic acid might be formed. Both malic and glycollic acids disappear rapidly from these aerated cultures, and in the first section of this discussion it has been pointed out that the simplest hypothesis to explain the disappearance of the glycollic acid is that it is converted into glycollic aldehyde which becomes polymerised to glycogen.

It appears to us then that the citric acid produced by cultures of *A. niger* may be involved in at least two types of reaction which are indicated below:

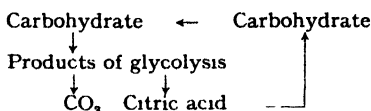


Under natural conditions, when the mycelium is well aerated, it would seem that the anabolic activities of the protoplast are so vigorous that all or almost all of the citric acid is involved in the first series of reactions. Under conditions of narcosis, such as may be induced by the relatively high concentrations of carbon dioxide in culture flasks tightly plugged with cotton-wool bungs, reactions of the second series evidently occur, as Challenger, Subramaniam, and Walker showed. The quantities of the products of these reactions obtained were not large, so that, even there, much of the citric acid may have taken part in reactions of type (I).

Active respiring cultures of the strain of *A. niger* which we used produce small accumulations of citric acid which disappear as the sugar concentration begins to decrease. This disappearance of the citric acid is prevented by the presence of calcium carbonate in the

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culture fluid, which becomes converted into calcium citrate, fairly large quantities of which may accumulate. The disappearance of the acid under ordinary circumstances is evidently due to its further conversion to other products, and the preceding discussion suggests that the most likely products are carbohydrates. One may therefore suggest the provisional scheme of reactions below as indicating the place occupied by citric acid in the respiratory processes of *A. niger* and possibly other mould fungi:



Somewhat similar evidence has suggested that the malic acid formed in many of the flowering plants has a very similar position in their respiratory processes (1, 3).

One should, in conclusion, point out that reconversion of citric acid to carbohydrate in the manner suggested is a process that appears to be analogous to the reconversion of lactic acid to glycogen in muscle (Meyerhof, Lohmann, and Meier(10)), and to the "oxidative anabolism" of Blackman(7).

APPENDIX

BY T. A. BENNET-CLARK

Since the joint work described above was concluded, further evidence has been obtained by one of us, which bears out the general conclusion that disappearance of citric acid and certain other organic acids is brought about by their reduction with eventual formation of carbohydrate. In this new work the oxygen consumption was recorded in addition to the carbon dioxide output. One typical experiment is described.

Culture A 13. Details of the methods used in determining the rates of the gas exchanges have been described(1), so it will suffice to point out that the atmosphere surrounding the culture is automatically kept constant in composition with an extremely low CO_2 tension and an oxygen tension equal to that of the air. The culture was grown in a tube 15 mm. in diameter on 5 per cent. glucose Czapek solution, 7 ml. of which were supplied.

After 150 hours the sugar solution was run out, the mycelium was washed and distilled water was replaced under sterile conditions.

During this period the course of CO_2 output was essentially similar to that which has already been described for the larger cultures of the active type. It will be noted that the R.Q. is greater than unity at the beginning, but decreases fairly quickly to unity. This high initial R.Q. is evidently due to the conversion of the oxygen-rich glucose and nitrate into oxygen-poor substances such as protein and lipoids. The mature mycelium maintains an R.Q. close to unity both during the phase when sugar supplies were available outside the fungus and during the starvation phase when water only was supplied.

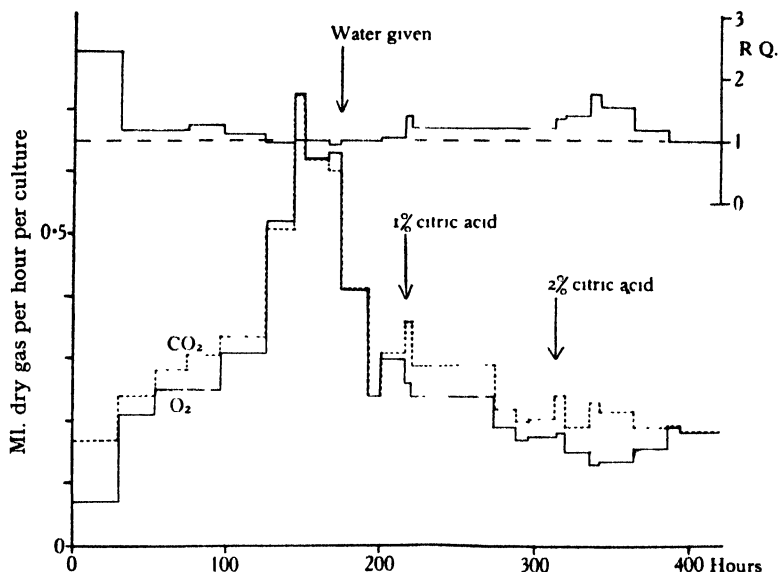


Fig. 10. Respiration-time curves of A13. Rate of CO_2 output ----, rate of oxygen intake ——. The R.Q. is given by the uppermost stepped record with ordinates on the right.

It is clear therefore that during the starvation phase carbohydrate (probably glycogen) is the substrate from which CO_2 is generated. It is improbable that protein decomposition products are produced during starvation which might neutralise citric or other acids supplied later. These results are graphically illustrated in Fig. 10. The gas exchanges are expressed as ml. of dry gas at N.T.P. per hour per culture. Variations in the R.Q. are given by the upper record.

At the time indicated by the second arrow the water was removed and replaced with 8.0 ml. of 0.143*N* citric acid. After 116 hours this citric acid was removed and the amount of acid left was determined.

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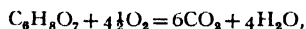
The quantity found was 42.0 mg., the initial amount was 73.0 mg., so 31.0 mg. of citric acid disappeared. The R.Q. rises above unity but does not attain very high values; data regarding the gas exchanges are tabulated below.

TABLE I

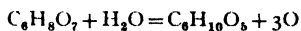
	First exposure	Second exposure
Citric acid lost (mg.)	31.0	26.6
Total CO ₂ evolved (ml. dry gas N.T.P.)	30.27	13.79
Total O ₂ absorbed (ml. dry gas N.T.P.)	26.02	9.96
O ₂ made available by reaction $C_6H_8O_7 - C_6H_{12}O_6 + 3O$ (ml. dry gas N.T.P.)	5.41	4.64
Total available O ₂ (ml. dry gas N.T.P.)	31.43	14.60
Gross R.Q.	0.967	0.943
Net R.Q.	1.162	1.383

At the time given by the third arrow in Fig. 10 (i.e. time of removal of the first dose of citric acid) a second dose was replaced, the concentration being 0.185*N*. After 106 hours it also was run out, and the citric acid loss was similarly determined. Data regarding this second exposure to citric acid are also given in Table I. In this case it will be noted (see Fig. 10) that the R.Q. rises considerably higher than unity, the maximum value attained being 1.75.

Oxidation of citric acid according to the empirical reaction



would result in a R.Q. of 1.33. The much larger R.Q. observed therefore indicates that the citric acid is at least partially being converted into a more reduced substance than itself. (It may be added in parenthesis that conversion of any substance into a more reduced substance would have the same effect on the value of the R.Q., for example, conversion of carbohydrate into alcohol or fat.) A reaction of the type required to explain the high R.Q. is our postulated conversion of citric acid to glycogen through the possible intermediate stage of glycollic aldehyde. This sequence of reactions may be formulated empirically as follows:



Oxygen is made available by these reactions and can reappear as part of the molecules of (CO₂) which are evolved. In the respiratory oxidation of sugars or products of glycolysis part of the oxygen used is according to our view generated by the above reactions, and part is absorbed from the atmosphere. The sum of these two quantities we call the total available oxygen.

If our hypothesis is correct evidently the "gross R.Q." (i.e. volume

CO₂ evolved/volume of total available oxygen) should equal unity. Table I indicates that this is in fact the case. The fact that the R.Q. exceeds the value of 1.33 is important confirmatory evidence in favour of the hypothesis that citric acid (or its hydrolysis or cleavage products) undergoes reduction probably to an aldehyde more especially since the value of the gross R.Q. is so close to unity.

Finally, the data of this experiment again rule out the possibility of oxidation of citric acid to carbon dioxide and water, as the figures of Table II show:

TABLE II

Citric acid loss	26.6 mg.
26.6 mg. citric acid on complete oxidation give CO ₂ output of	18.3 ml.
CO ₂ output observed	13.79 ml.
26.6 mg. citric acid require for complete oxidation O ₂ intake of	13.8 ml.
O ₂ intake observed	9.96 ml.

The observed gas exchanges substantiate the results referring to carbon dioxide output only, which are dealt with in the first part of this paper.

SUMMARY

1. The respiration of a strain of *Aspergillus niger* has been investigated, and it is shown that starvation (i.e. removal of the external sugar supply) causes the respiration rate to fall to a low value, the starvation rate. Long periods of starvation do not have a permanently injurious effect on the fungus, as it is found that on resupply of the original culture solution the respiration rate recovers to its original value, the "floating respiration" rate.

2. When the starving fungus is supplied with citric, malic, glycollic, or oxalic acids instead of glucose the rate of CO₂ output remains unchanged at the "starvation level". This CO₂ is almost certainly produced from reserve polysaccharide, so that no CO₂ arises from the acid supplied. Notwithstanding this, acid disappears rapidly from the culture fluid. The acid-C loss commonly exceeds the CO₂-C output.

3. Neutralisation of the acid is shown to be impossible, and, as certain other possibilities are eliminated also, one concludes that loss of acidity is due to reduction of carboxyl to carbonyl. We assume provisionally that the hydroxyaldehydes so formed are polymerised to polysaccharide. The process appears to be somewhat similar to the oxidative anabolism of Blackman or the Meyerhof cycle.

4. Data of oxygen intake are also in agreement with this view, and it is also shown that during the phase of rapid disappearance of

Utilisation of Organic Acids by Aspergillus niger 231

citric acid the value of the R.Q. considerably exceeds 1.33, which is the highest value that could be attained by simple oxidation of the acid. This high value (1.75) suggests that the citric acid (or its cleavage products) is reduced and not oxidised by the mycelium.

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THE OVULAR APPARATUS OF *SPHENOPTERIDIUM AFFINE* AND *BIFIDUM* AND OF *DIPLOPTERIDIUM* (*SPHENOPTERIDIUM*) *TEILIANUM* (WALTON)

By M. BENSON

(With Plates V and VI and 5 figures in the text)

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INTRODUCTION

THE types now found to represent the ovular apparatus of *Sphenopteridium affine* and *bifidum* have for long borne the names of *Telangium affine* and *Telangium bifidum*, as they were regarded as Synangia of the type of *Telangium Scotti* Benson(1). A more detailed investigation of the specimens has shown they are ovular. It is the object of this paper to state what observations have been made and to show that they definitely indicate that we must transfer these types from the form-genus *Telangium* to that of *Calathiops* Goeppert, emend. The revised diagnosis runs as follows:

"Naked, fertile pinna presumed to belong to a Pteridosperm. The pinna bears pedicellate, cupulate ovules which, when immature, are crowded together on the more or less sympodially produced ultimate arms. When mature (i.e. ready for pollination) the ovules escape from the cupules while the pedicels are still short as in *Calathiops Bernhardtii*(6) or occasionally, after their elongation, which results in a Calymmatotheca-like fructification."

After perusal of this paper it will, I believe, be obvious to the reader that *Telangium affine* and *bifidum*, and *Teilanium* must be

included in the form genus *Calathiops* Goeppert, emend., and I will therefore refer to them throughout under their new names:

Telangium affine = *Calathiops affinis*,

Telangium bifidum = *Calathiops bifida*.

The ovular apparatus of

Diplopteridium Teilanum = *Calathiops Teiliana*.

One other type to which reference is made, but which cannot so far be attributed to *Sphenopteridium*, is *Schuetzia Bennieana* (Kidston spec.). This has been shown by Dr Halle to resemble closely the above forms, the only distinction being the spicate branching of the pinna. The generic name is in urgent need of change, as the term *Schuetzia* Schuster⁽¹²⁾ was applied to two distinct types. I propose therefore to include this type also in *Calathiops* and to refer to it under the name *Ca. Bennieana*.

The specific name is in all the four cases retained unchanged.

GENERAL COMPARATIVE ACCOUNT OF *CALATHIOPS AFFINIS*

AND *BIFIDA*

(1) *Calathiops affinis*

These incrustations (Text-fig. 1) are abundant in the Westphalian (Yorkshire) oil-shales, and collections were made especially by the late C. W. Peach⁽¹¹⁾. In these the tuft-like bodies, long regarded as synangia, were found on naked, bifurcating pinnae which Peach suggested were parasitic like Dodder on the frond of *Sphenopteridium affine*. There is no frond now preserved showing this continuity with the ovuliferous pinna, although such continuity is always assumed. Kidston, one would think, must have had proof of it, as he always used the same specific name for the frond and the *Calathiops* tuft⁽¹⁰⁾.

The three museum specimens to which I specially wish to refer are at differing stages of maturity. The two younger fructifications are in the Geological Survey Museum, London, forming part of the Kidston Collection under the numbers 627 and 637, and the third is in the Geological Department (British Museum) under the number V. 4231.

The characters used are:

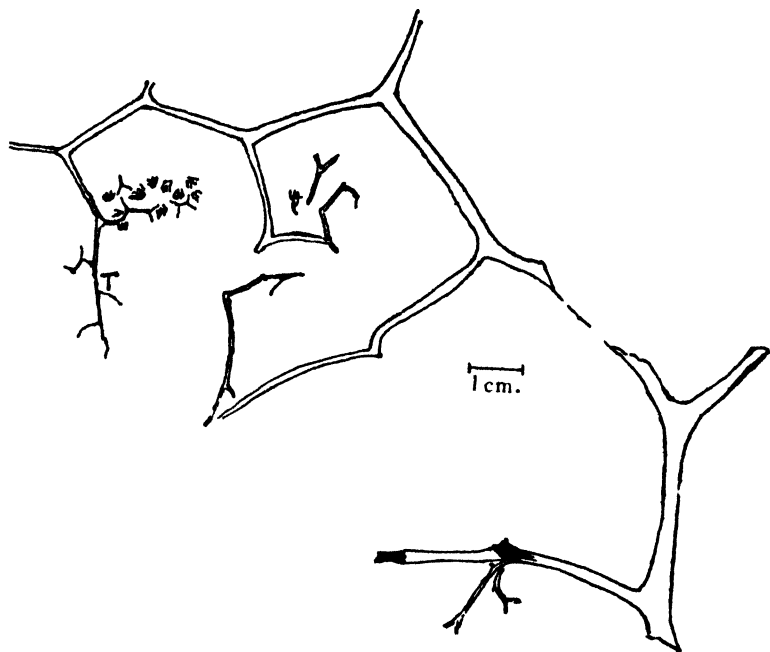
- (1) Method of branching of the pinnae.
- (2) Potential number of ultimate units.
- (3) Size and character of the bodies on the terminals.



Text-fig. 1. A drawing from the stone of a fine specimen of the ovular pinna of *Sphenopteridium affine* showing the mode of branching and at *T*, *T*, the pairs of "terminal systems" with less regular dichotomy. The buds are in an early phase of disintegration but not so advanced as those in Text-fig. 2. In the topmost bud the γ tufts are still intact. Kidston has an enlarged photograph of these buds in Pl. CIV, fig. 5(10).

All three specimens (Text-figs. 1, 2 and 3) have the same characteristic mode of branching.

Almost regular, equal dichotomy at an angle of 120° is repeated from six to eight times and is then succeeded by paired "terminal systems" of very delicate short-armed forks (Text-fig. 2 *a*, *TT*). These show, moreover, a tendency to unilateral dichotomy, so that in these "terminal systems" the calculation of the number of potential



Text-fig. 2. Kidston's Text-fig. 43 of an older phase in which the ultimate terminals in some cases bear single ovules of which probably over fifty occurred on one "system". This drawing should be compared with the new photograph from the same specimen in Pl. V, fig 3, and with Text-fig. 2 *a* and *b*.

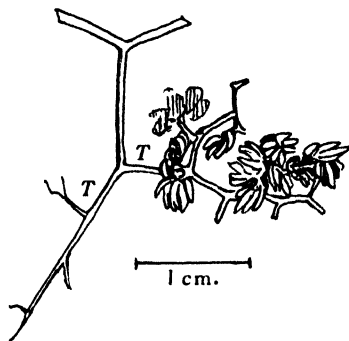
terminals by applying the Law of Geometric Progression breaks down.

On the terminals occur the *Calathiops* buds or their residual constituents, whether ovules or empty cupules. On each of the "terminal systems" (Text-figs. 1-2 *a*, *T*) there are about five dichotomies, thus immensely increasing the number of ultimate terminals. It is probable that the initiation of these systems heralds the final breaking up of the *Calathiops* tuft in *Sphenopteridium affine*, and the

pair seems comparable with the sympodial *Whittleseya*-like seed-bearing body in *Calathiops Bernhardti* (6).

Let us take the three specimens in order and begin with 627, which is the most immature (Text-fig. 1). The systems *TT* in this figure are only half complete and the tufts only partially disintegrated, so that we cannot count their constituent units. The drawing has been made from the stone, as a better exposure of the branching has been achieved since Kidston's fine photograph was taken (*vide* Pl. CI, fig. 1 and Pl. CIV, fig. 5 (10)).

In another specimen in the Kidston Collection (637) the detail of a "terminal system" *T* has been most successfully photographed by Mr Tams (*vide* Pl. V, fig. 3 and Text-fig. 2 *a*) so that we can study a later stage of development. The system is a nearly complete one and

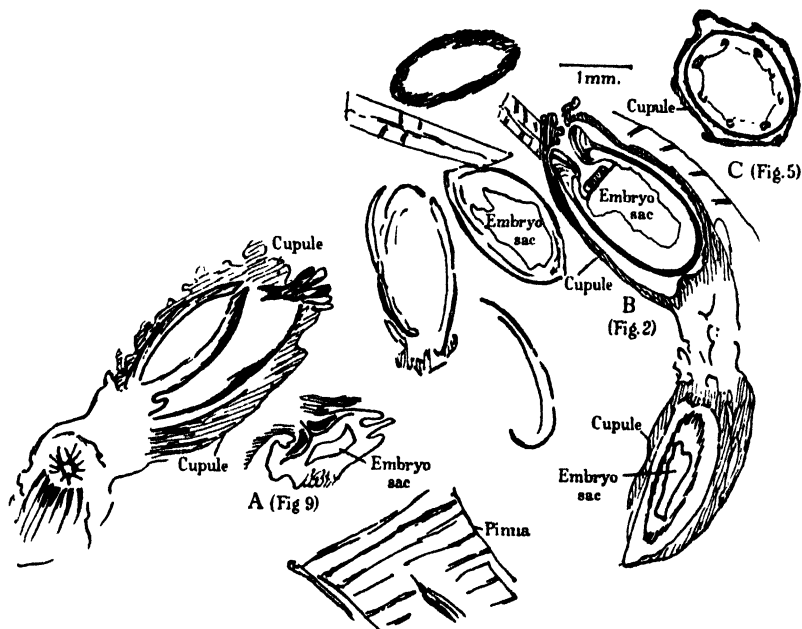


Text-fig. 2 *a*. *Calathiops affinis*. Detail for the explanation of the residual bodies on a "terminal system" in Text-fig. 2

the young cupulate ovules are now for a large part separated out from their compressed condition and are seated, one to each of the ultimate forks of the rachis. In this specimen they are just the size of the young *Sphaerostoma* ovules figured in the description of that seed on Pl. I (2), and we may surmise that before they were shed the cupules would have lengthened. These tufts appear to be at the same penultimate stage of disintegration as that shown in section on the slide Scott Coll. C.N. 387, at the British Museum. A map of this remarkable slide is given in Text-fig. 2 *b*.

The third stage available is the specimen in the Geological Department of the British Museum, South Kensington, labelled V. 4231. This has been photographed by Mr Herring (Pl. V, fig. 2) and a map is given in Text-fig. 3. This large specimen, measuring $6 \times 5\frac{1}{2}$ in. in length and breadth, has shed all its seeds and almost all

its cupules. We can, however, count about eight systems of delicate terminals *T* on the one half of the pinna, so that if each bore quite fifty seeds as in the previous specimen, the ovules may have been at least 800 in number and probably far more.



Text-fig. 2 b *Sphaerostoma ovale* A drawing from a section (Scott Coll. British Museum, C.N. 387) which is regarded as passing through a partially disintegrated *Calathiops* bud in much the same stage of development as that of Text-fig. 2 a. This section is the only one known which shows *Sphaerostoma* in a cupular envelope. The cupules are much more delicate in structure than those of *Calathiops Bernhardi* (6), and it is probable that in this respect *Heterangium* and *Sphenopteridium* seeds resembled one another. A, B, C refer to figures in Benson (2).

(2) *Calathiops bifida*

In these specimens we may study the structure of single buds and also final stages of the shedding of the ovules from their cupules. Moreover, it was from Kidston's specimen (*vide* Pl. CIII, fig. 6(10)) that Halle obtained the specimen he figures in Pl. XV, figs. 8-12(9). Kidston's figure shows the buds were about 3 mm. in height and 2.6 mm. in width. They were composed of four aggregates. Thus, if the whole bud be called an α tuft, each of these four may be called a β tuft. Further, each β tuft is composed of about five minor

aggregates or γ tufts of young cupulate ovules, with nothing preserved but their cuticularised membranes and embryo sacs.

If we turn to Kidston's Pl. CII, figs. 5 and 6 (10), we see four bodies which Kidston calls synangia (i.e. α tufts) spread out and seen from below. If each is an α tuft we see the β tufts uniformly separated



Text-fig. 3. An explanatory drawing from the stone of the largest of Peach's specimens of *Sphenopteridium affine*. Almost all the cupules and seeds have perished.

into their constituent parts, i.e. γ tufts, and each γ tuft looks like a black finger. Each γ tuft is about 2 mm. in length and this is its length in Pl. CIII, fig. 8, where it is called a sporange. All the *Ca. bifida* buds, or α tufts, so far considered are in an early phase.

Let us turn next to the specimen shown in Kidston's Text-fig. 44, p. 457 (10), and compare the tufts with those in Pl. CIII, figs. 3 and 4, which show bodies approximately of the same size as one another

but all much larger than those in Pl. CII, figs. 5 and 6, and those in Halle's figures, Pl. XV, figs. 10-12(9).

Of the three groups those in Kidston's Pl. CIII, fig. 4(10), are slightly the largest. These appear to consist of single empty cupules which have shed their seeds and would thus be of the maximum dimensions for the species. Those of fig. 3 may not have quite reached the ultimate phase. Those in Kidston's Text-fig. 44(10) are obviously almost at their maximum size. They must have been attached (if they belong to this frond of *Sphenopteridium bifidum*) somewhat peripherally on the forking pinna, but this gives no clue to the number of bifurcations.

We are thus limited in the use we make of *Ca. bifida* specimens for comparative purposes, since it is not quite clear that the pinna-bearing cupules in Text-fig. 44(10) actually belonged to the frond, nor do we know the evidence Kidston relied on for stating Pl. CIV, fig. 6(10) shows bodies safely to be attributed to *Sphenopteridium bifidum*. The arguments, however, to be drawn from the consideration of series in definite species is perfectly sound as regards *Sphenopteridium affine* and considerably supplemented by one other case which is dealt with later.

ANATOMICAL STRUCTURE OF *CALATHIOPS BIFIDA*

The anatomical structure of *Ca. bifida* was determined from Halle's sections now in the Geological Survey Museum, London. Halle's method of treating the incrustated plant remains after removal from the rock was described in his important treatise(9), and it is by careful study of some of the microtome sections made by him that the following results have been secured. The following sections are figured:

Slide 3461 (Pl. VI, fig. 5), high-power micrograph of part of I. 3.

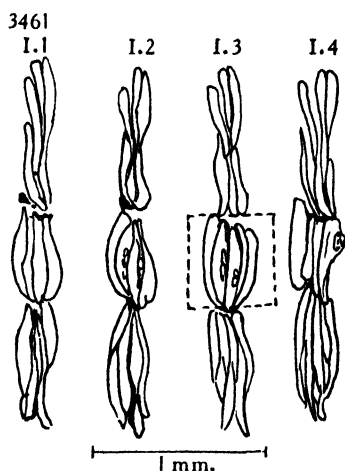
Slide 3461 (Text-fig. 4), low-power drawing of I. 1-4.

Slide 534 (Text-fig. 5), camera drawing of cuticle and cells.

Text-fig. 4 shows the arrangement of the aggregates (β and γ tufts), the latter appearing somewhat crescentic in transverse section. The portion enclosed in dots is that represented in the micrograph Pl. VI, fig. 5. This fig. 5 exhibits the contents of the γ tufts. They consist of compressed cuticles which enfold numerous embryo sacs.

The embryo sacs

Each embryo sac may be represented in section in two or even three successive microtome sections. They were probably rounded. (They appear in some preparations kindly made for me by Dr Dix like yellow discs.) A very remarkable observation has been made in Halle's slides of granular contents in strands with denser bodies enclosed. Anyone with the slide well illuminated under the high power of the microscope would be inclined to identify these contents as protoplasmic strands containing nuclei.



Text-fig. 4. Four successive microtome sections transverse of an α tuft of *Ca. bifida* from Halle's slide 3461 now at the Geological Survey Museum, London. It shows that the same embryo sac appears in I, 2 and 3, and indicates the part of the section I, 3, enlarged on the Pl VI, fig. 5. The embryo sac indicated in I, 4 should be examined in the original slide for contents.

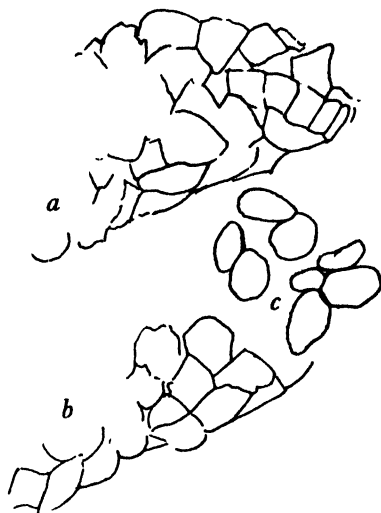
That anything of this nature could have survived solution after Halle's drastic treatment in softening the incrustation can only be explained by the fact that the cuticle of the embryo sac at this early stage is intact, and since the cuticle has not perished it seems that the contents are also preserved.

Neither in Dr Dix's nor in Dr Halle's preparations have I been able to detect a persistent triradiate scar such as Kidston revealed in the *Ca. Bennieana* he examined and figured on Pl. CVII, fig. 13 (10), but I have not had many free ones under observation. In any case a Y scar on an embryo sac is probably only very temporarily preserved. These Y scars on the walls of young embryo sacs indicate

that the latter must have arisen from spores of a triradiate type, some few of which have been found in *Ca. Bennieana* (4).

In the *Ca. bifida* α tuft cut by Halle there are undoubtedly some hundreds of embryo sacs (they are not all entered in Text-fig. 4).

This observation, which seemed baffling at first, proves to support and explain the results arrived at in the earlier part of this paper, viz. that there must have been quite 800 terminals before the branching of the *Ca. affinis* was complete.



Text-fig. 5 *a*, *b* and *c*. *a* and *b* are surface views of an α tuft showing the outline of the epidermal cells on the cuticle of the cupular lobes *c* shows camera drawing of the cells when free (they are a little over 40μ in average linear dimension).

The cuticles

Text-fig. 5 *a* and *b* are camera drawings from the thin margin and apex of a γ tuft of *Ca. bifida* when mounted whole as in Pl. V, fig. 1. The actual γ tuft drawn occurred in slide 534, and from that were drawn the oval free bodies which escaped from the tuft and are given in Text-fig. 5 *c*. They have been dissolved from the wall and may be looked upon most certainly as of vegetative nature. If one examines closely the transverse section of the β tuft in Pl. VI, fig. 5, there appear to be pairs of parallel lines. There are several possible explanations of this appearance. The inner line may be due to the roofs of epidermal cells shown in Text-fig. 5. On the other hand, it may be

due to stratified layers of cutin. This would render the walls less resistant to some reagents and explain Dr Dix's results. In the preparations made with Ashby's Cellulose-Film Transfer Method (see Walton, *Congrès Strat. Carbon.*, Heerlen, 1928, p. 753) the cuticularised walls of the epidermal cells tended to be dissolved, while those of the disc-like embryo sacs remained intact although somewhat swollen. These results possibly explain some of the difficulties Kidston met with in interpreting the *Ca. bifida* tuft.

GENERAL COMPARATIVE ACCOUNT OF THREE SPECIMENS OF
CALATHIOPS TEILIANA

Further light is thrown on the ontogeny of the ovular pinnae by a consideration of those found to belong to this third Sphenopteridian species.

The collections I made at Teilia in 1933 included a fertile frond younger than that elucidated by Walton (13). The *Calathiops* buds are 6.5 mm. in height and 5 mm. in width, and are borne on a pinna which probably had forked but thrice (Pl. VI, fig. 6). From this we may assume there were but eight buds of which six only are exposed on the plane of cleavage of the limestone block.

In the Shone specimen at the Chester Museum the buds are smaller, and Prof. Walton was able to demonstrate that the fertile pinna had forked six times. Therefore there must have been at least sixty-four buds. It is probable, therefore, that the buds in the former specimen (Pl. VI, fig. 6) contained eight times the number of ovules in the latter.

A third specimen is figured (Pl. VI, fig. 7) showing the basal forks of a fertile pinna, but the peripheral parts are buried in the rock. It shows the fertile, naked pinna fallen over as probably generally happened before the seeds were shed.

Incidentally this British Museum specimen (V. 2847, Pl. VI, fig. 7) is interesting as showing the fertile pinna as itself the result of the dichotomy of the right-hand sterile primary pinna. This seems to indicate that the frond, though appearing to be pinnate, i.e. having a terminal prolongation of the main rachis, is best regarded as derived from a dichotomising type.

SUMMARY

In this paper an attempt has been made to discriminate the phases of the different specimens of *Ca. affinis* and *bifida*, and of *Ca. Teiliana* (three species of ovular apparatus of the Lower Carboni-

ferous Sphenopterideae); and to show that the prong-like structures composing the body (see Pl. V, fig. 1) are tufts of young ovules of which all has perished except the cuticularised parts.

Also it is shown that Kidston's figures (Pl. CIII, 3 and 4⁽¹⁰⁾) represent empty cupules, the lobes of which have not been hitherto distinguished from the tufts of ovules in more immature structures, e.g. Pl. CII, fig. 6⁽¹⁰⁾.

In fact not only have these two types of body been confused with one another in Kidston's Memoirs, but each was identified with a pollen sac. Thus a single cupule with its six lobes, and an aggregate of probably 4×4 or sixteen ovules in their skeletal condition were each regarded as a pollen synangium.

The result of the investigation is that we now transfer these reproductive bodies from the form-genus *Telangium* to that of *Calathiops* Goeppert, emend.⁽⁶⁾ Meanwhile their specific names are retained.

I desire to express grateful acknowledgment of my indebtedness to the following: Dr T. G. Halle for the use of his unique slides and for the loan of photographs. Prof. W. Gothan for the loan of his magnificent specimen of *Calathiops Bernhardtii*⁽⁶⁾. Mr W. N. Edwards and Dr Crookall for their invariable and valuable assistance in the study of the specimens respectively in their charge in the Geological Department of the British Museum, South Kensington, and in the Kidston Collection in the Geological Survey Museum, London. Dr Emily Dix for most kindly undertaking at my request special preparations of *Ca. bifida* for this work as described in the text.

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EXPLANATION OF PLATES V AND VI

(All figures are untouched photographs. Fig. 1 is loaned by Dr Halle. Fig. 2 was taken by Mr Herring and Figs. 3-7 by Mr Tams.)

PLATE V

- Fig. 1. An α tuft of *Calathrops bifida* removed from the stone and mounted whole. The white spots probably indicate embryo sacs. $\times 12$.
 Fig. 2. A fine photograph from the stone of the largest of Peach's specimens of *Ca. affinis*. The specimen is in the Geological Department of the British Museum, South Kensington, under the registered number V. 4231. Text-fig 3 shows more clearly the "terminal systems" which require a lens in the photograph. $\times \frac{2}{3}$.
 Fig. 3. An excellent photograph of the detail of a single "terminal system" from specimen 637, of which part is given in fig 4. A map is given in Text-fig 2 a. A simple calculation can be made showing that at least fifty ovules would have been present if complete. Comparison should be made with Text-fig 2 b which is provided to show that in *Sphaerostoma* (Scott Coll. C N. 387 at the British Museum) the cupular envelopes were similar to those in *Sphenopteridium affine* and not long rigid bodies as in *Ca. Bernhardtii* (6) $\times 3$.

PLATE VI

- Fig. 4. *Ca. affinis*. Part of an ovular pinna (Geological Survey Museum, Kidston Coll. 637) from which Pl. V, fig 3, was taken. $\times \frac{2}{3}$.
 Fig. 5. A micrograph from one of Halle's sections (the portion in Text-fig 4 which is outlined with dots). Description in text. $\times 200$.
 Fig. 6. One of the counterparts of a new specimen of *Diplopteridium* with associated *Ca. Teihana*. The attachment of the base of the ovular pinna is seen better in the other counterpart. A piece of the forking distal part can be seen slightly to the left of the two groups of buds. Natural size.
 Fig. 7. A fertile frond of *Diplopteridium Teihanum* (British Museum, V. 2847) with forking pinna attached as an arm of the right-hand pinna. Five forks are seen but the terminals are immersed in the stone. Very slightly enlarged.



Fig. 1

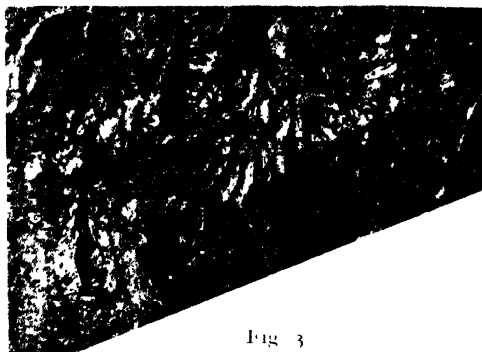


Fig. 3

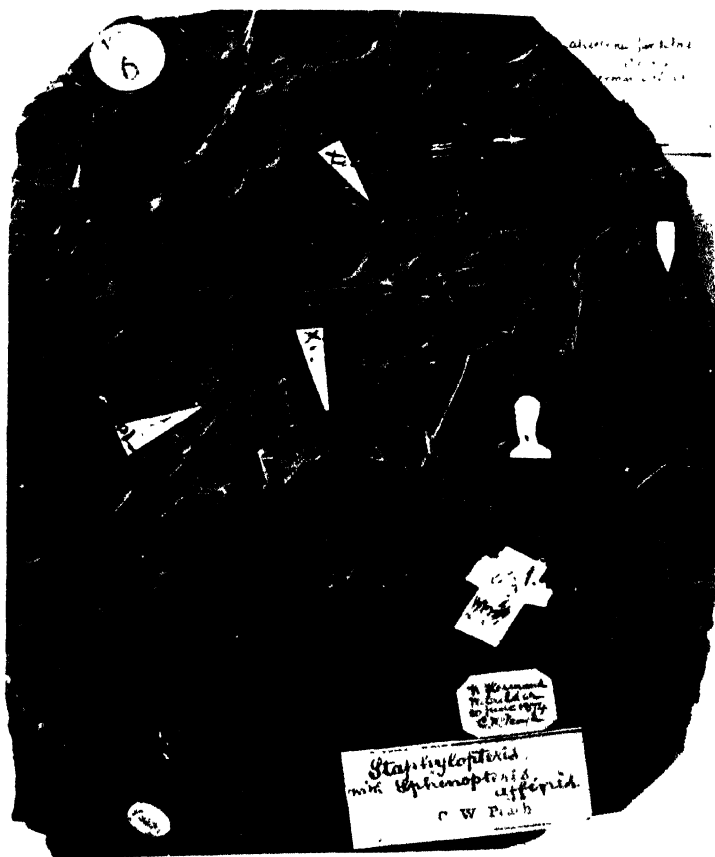


Fig. 2

BENSON—OVULAR APPARATUS OF *SPHENOPTERIDIUM AFFINE*

LIBERATION OF OOGONIA IN *BIFURCARIA* AND OTHER MEMBERS OF THE FUCACEAE

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(With 8 figures in the text)

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I. INTRODUCTION

MOST of the Fucaceae are surf-loving plants of definitely intertidal habit. Extreme contrasts are seen in the European *Pelvetia* which can survive on rocks above high-water mark, only occasionally reached by spray, and by such a plant as the South African *Fucus constrictus* Harv.¹ living in deep water well below the intertidal zone. The great majority, however, are emergent, either occupying rock surfaces which are exposed when the tide recedes (*Fucus*, *Ascophyllum*, *Himanthalia*) or inhabiting rock pools. In shallow pools they may be migrants from a typical rock habitat, the fronds being partly exposed; but in deeper pools near low-water mark occur fucaceous plants (*Cystoseira*, *Halidrys*, *Sargassum*), which are rarely uncovered and indeed are hardly accessible except at low spring tides.

The oogonium of the Fucaceae encloses one or more oospheres, and the wall is differentiated into an outer very thin layer, the so-called "exochiton", and an inner gelatinous region, which is sometimes visibly separated from the former (Farmer and Williams, 1898). When maturity is reached, the exochiton ruptures at the apex,

¹ Gruber describes this as "*Axillaria constricta*" (1896), but de Toni lists it as *Ascophyllum? constrictum* (1895).

releasing the contents completely enclosed by the swollen inner wall. This release of apparently whole oogonia¹ (and antheridia) from the ruptured exochiton appears to be a feature of all fucaceous plants. The exochiton usually persists *in situ* for some time as a delicate membranous cup, recalling the empty shell left after dehiscence of zoospores and gametes in the lower brown algae.

The oogonial contents are liberated first into the cavity of the conceptacle and are subsequently extruded into as a green mucilaginous drop at first adherent to the osteole; the mechanism of the extrusion is not, however, fully understood. Liberation may be observed when a fertile frond with ripe conceptacles is left to dry for a few hours. It is commonly accepted that on exposure, contraction of the cortex causes the osteoles to gape widely and exerts pressure on the mucilage from the paraphyses; this view is based presumably on the statement of Farmer and Williams (1898), but these authors add that "this expulsion is reinforced by the swelling of the oogonial walls when ripe oogonia are available". Pierce and Randolph (1905) found that extrusion was greatly accelerated by light, and state that maximum extrusion occurs in nature during the early morning hours. Schreiber² (1930) claims that although there is some extrusion in *Fucus serratus* during low water, as believed by Thuret, Oltmanns and others, it is much greater on subsequent wetting with fresh water or sea water, thus after the return of the tide. Oltmanns notes that release of oogonia can occur in *Fucus* in spite of continued submergence, so that neither partial drying nor the subsequent absorption of water can be the whole explanation.

A modification of what may be called "emergent" fertilisation occurs in the genus *Pelvetia*, for here the oospheres are permanently enclosed after extrusion from the exochiton. The plant is hermaphrodite and fertilisation occurs by penetration of the mucilaginous membrane, within which germination of the oospore also takes place—a method obviously suited to the almost terrestrial conditions under which *Pelvetia* can survive. In *Fucus spiralis*, also hermaphrodite and occurring relatively high up on the shore, not only extrusion but also fertilisation can apparently occur during the ebb, for when Kniep (1907) left receptacles with ripe oogonia to dry under a bell

¹ The use of the word "oogonium" for the extruded contents of the oogonium as well as for the original structure is confusing, but appears to be firmly established.

² Schreiber does not appear to distinguish between the extrusion of the entire oogonial mass (apart from exochiton) and the naked oospheres which escape from the inner layers of the oogonial walls and function as gametes.

glass over night, and examined them next day, he found early stages of germination of free oospores.

A very different type of extrusion has been described for *Sargassum* and *Cystophyllum* on the coast of Japan. Here the extruded oogonia remain for at least two or three days firmly attached to the interior of the conceptacle by means of gelatinous stalks. The extrusion is periodic, occurring in *Sargassum encrue* one day and in *S. Horneri* three days after the full moon. The lower part of the receptacle ripens first, and after discharge the upper part of the receptacle becomes involved (Fig. 1). In *Cystophyllum sisymbrioides* a similar production

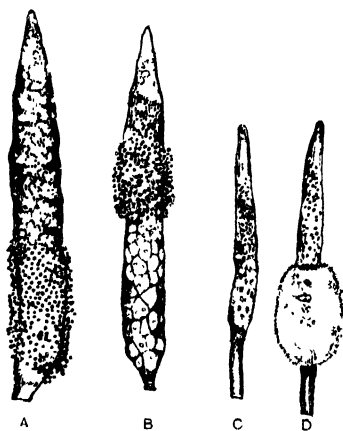


Fig. 1. A, B, receptacles of *Sargassum Horneri* Ag., A with the lower, B with the upper conceptacles bearing attached oogonia, the lower part of B has spent conceptacles from which the sporelings have dropped off. C, D, receptacles of *Cystophyllum sisymbrioides*, in C the lower conceptacles have spent their oogonia; in D the lower part has oogonia among the paraphyses (After Tahara, 1913.)

of gelatinous stalks was found, but here the paraphyses push their way through before the oogonia appear. The paraphyses are at first stiff, but become soft after extrusion and form a slimy matrix in which the oogonia are enclosed (Fig. 1 C, D). In both, sporelings in various stages were found held to the parent plant by the enveloping mucilage.

More recently Kunieda (1926) traced the origin of these stalks in *Sargassum Horneri* to the modification of the oogonial wall (presumably the middle layer) which thickens, forming a sort of gelatinous cap over the part of the oogonium facing the cavity of the conceptacle. This cap swells and extends into a stalk, one end of which remains

attached to the oogonium which is dragged out of the conceptacle in an inverted position while the other end becomes attached to the interior of the conceptacle (Fig. 2). It is not clear from the description what becomes of the exochiton, which from the figure appears to have been torn and ruptured beyond recognition.

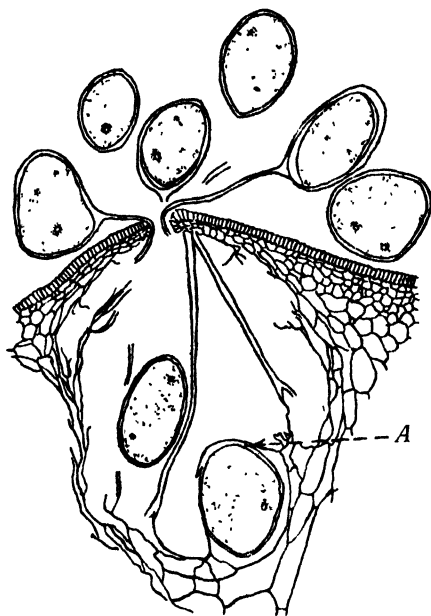


Fig. 2. *Sargassum Horneri* Ag. Diagram reconstructed from three sections showing oogonia attached to the conceptacle. At A the "gelatinous cap" is seen. (After Kunieda, 1928.)

The following observations suggest that the habit of attaching the oogonia to the parent plant for a time may be more widespread among the Fucaceae than has been suspected.

II. MATERIAL AND METHODS

Only dried material was available for most of the types examined, but material preserved in formalin was also used for *Bifurcaria brassiciformis* and *Sargassum lendigerum*. A large number of herbarium specimens have been examined, but most of them were useless for the present purpose, being sterile, male or female, with the conceptacles in an unsuitable stage of development. Only rarely, dried oogonia could be detected in close association with the osteoles, and on cautious soaking with sea water or with lactic phenol could be

seen suspended by gelatinous threads. For more resistant material it was sometimes necessary to boil for a minute with lactic phenol (diluted to about 50 per cent.) to expel air. Hand sections through such material show an occasional oogonium attached to the receptacle by a stout stalk not much longer than the depth of the conceptacle, but they are apt to become detached, unless the greatest care is exercised in handling. The mucilage takes up freshly made gentian violet better than most stains, but the affinity for it depends greatly on the degree and possibly the manner of hydration which has been achieved.

III. OBSERVATIONS ON *BIFURCARIA BRASSICIFORMIS* STACK. Ktz.

In 1920, the writer had the opportunity of collecting material of *Bifurcaria brassiciformis*¹ from various localities along the shores of the Cape Peninsula, and whilst examining fresh fronds it was noticed that the oogonia extruded from the conceptacles were difficult to remove with needles and were in fact firmly attached to the mouths of the conceptacles by tough gelatinous stalks. By moistening with sea water a fertile shoot which had been exposed for a short time to the air, the whole contents of the conceptacle were forcibly ejected and the oogonia could be seen carried up on stalks which were themselves embedded in a tenacious common jelly, presumably derived from the walls of the paraphyses. On older shoots the jelly was much less tenacious, and many of the stalks had lost their oogonia; a number of oospheres and also sporelings could be seen entangled among the now headless stalks.² A short account of these observations was given to Section K at the meeting of the British Association in 1925.

The three species of *Bifurcaria* are characteristically found on rocky shores in the lower stretches of the intertidal zone. In the Cape Peninsula, *B. brassiciformis* frequently fills narrow creeks between rocks where the water remained a foot or more in depth even at the lowest spring tides. Like the British species, *B. brassiciformis* has a creeping much-branched rhizomatous base adherent to the rock face by many small sucker-like discs, and sends up numerous cylindrical

¹ The name *Bifurcaria* was first applied by Stackhouse to a plant described as *B. tuberculata* in 1809. In 1843, Kutzing described three species under the name *Pycnophycus*, distinguishing the two South African species as *P. brassiciformis* and *P. laevigata* respectively, but retaining the specific name *tuberculatus* for the species previously described by Stackhouse. Thus on grounds of priority, the nomenclature should be *Bifurcaria brassiciformis*.

² Young plants of macroscopic size were, however, not found, though often sought.

fronds, the fertile regions of which are always submerged, excepting the extreme tips at low water. Unlike the British species, the conceptacles are unisexual and the plant more or less dioecious, both sexes being abundant in November and December, the Cape spring time. Owing to the strongly social habit, with spreading much-branched rhizome, it is not easy to determine how far one individual extends, but some branches bear entirely (or almost entirely) female conceptacles and others appear to be entirely male in development.

The fertile tips are flattened, bearing the conceptacles arranged in a vertical row along either edge (Fig. 3), the youngest nearest the growing point. At dehiscence, each osteole is surmounted by a glistening drop of mucilage in which the oogonia are visible to the naked eye. The ejection is perhaps periodic, for very young oogonia are to be seen in conceptacles which have already extruded a large number of mature oogonia. In Fig. 4 three nearly full-grown oogonia show stages in the development of an apical cap of mucilage. It appears that a plate or series of plates of denser mucilage form just above the oosphere, beneath the exochiton. Some of these separate and swell as the mucilage expands into a stalk, but beneath the extruded oogonia others are still sometimes visible. On mounting such oogonia in lactic phenol, these plates are seen to swell, the mucilage still stretching between them, until finally only a structureless but tenacious strand remains. This extension on swelling was described in a general account of the whole question given to the Linnean Society (Delf, 1930),¹ but the developmental stages had not then been seen.

When the oosphere is ripe, the accumulated mucilage above it expands, pushing aside a cap of exochiton and curving over towards



Fig. 3 Single' receptacle of *Bifurcaria brassyiformis* Ktz., with attached oogonia, slightly magnified. The dotted areas indicate the extent of the conceptacles in various stages of their development. In the lowest part the mucilage has dispersed and the sporelings dropped away.

¹ In a chance conversation with Dr Knight some time later, she informed me that she had also examined material of this plant and had already followed the development of the oogonial stalks from the mucilage formed in the upper part of the oogonium.

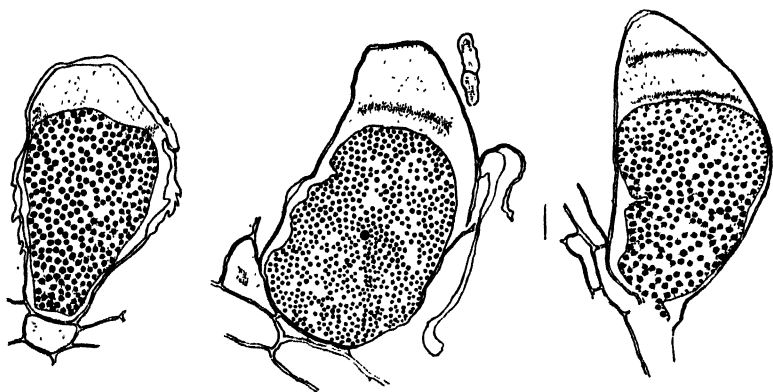


Fig 4. Single oogonia of *Bifurcaria brassiciformis* Ktz., showing the development of the gelatinous cap above the oosphere. From a conceptacle with nearly ripe oogonia. (Drawings by Mrs F. Laing)

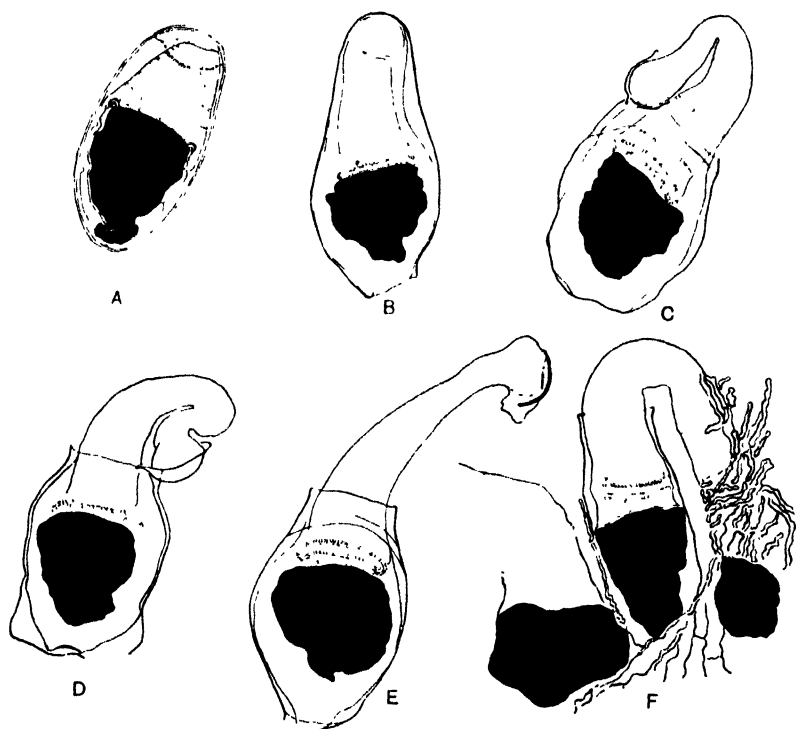


Fig. 5. Oogonia of *Bifurcaria brassiciformis* Ktz., from a slightly older conceptacle, showing stages in the extrusion of the gelatinous stalk. The free upper part of the gelatinous stalk has carried away a cap of exochiton. In (F) the free end is firmly held by a mass of adjoining paraphyses. (Drawings by Mrs F. Laing.)

the wall of the conceptacle (Fig. 5). With further expansion, the stalk straightens, the oosphere (surrounded by endochiton and probably also by mesochiton) is withdrawn, inverted, and owing to mutual pressure is thrust out of the osteole. The mucilaginous plates perhaps give an added elasticity during the tension of withdrawal or may allow of adjustment in length, the more deeply placed oogonia needing longer stalks than those in the neighbourhood of the osteole. A large number are extruded at once, about 250 from one conceptacle. In spent conceptacles, the cup-like empty exochiton of the oogonia can often be seen after staining still attached to the basal cell, but in my material they are very resistant to the ordinary stains for mucilaginous structures and are almost invisible without some coloration. The exochiton is sometimes severely ruptured and the shreds collapse, so that it can scarcely be recognised. The top of the exochiton cup sometimes remains in connection with one end of the gelatinous stalk (Fig. 5 C, D), but more often the latter seems to be independently fixed to the inner wall of the conceptacle as in Fig. 5 E; it must be remembered, however, that the latter is derived from herbarium material in which some displacement may have occurred.

IV. OOGONIAL RELEASE IN *SARGASSUM*

Fifteen species of *Sargassum* have been examined by the writer in herbarium material from China, Japan and South Africa. Attached oogonia or sporelings have been detected in three species.

(a) *Sargassum lendigerum* Turn. Ktz.

Good preservation was found in a specimen gathered for me at Umkomaas (Natal) in December 1920 and roughly dried without much pressure. Similar material collected in November 1928 and preserved in formation was kindly sent by Miss E. L. Stephens of the University of Cape Town. Thick hand sections through a receptacle such as Fig. 6 A show the oogonium enveloped in mucilage and attached by a stalk ending in a refractive disc which is probably part of the exochiton torn away (Fig. 6 B). These oogonia may often be dissected away intact (Fig. 6 C), but in old, empty conceptacles the broken base of a stalk can sometimes be seen close against the inner wall of the conceptacle, showing how firmly the oogonia can be held in position.

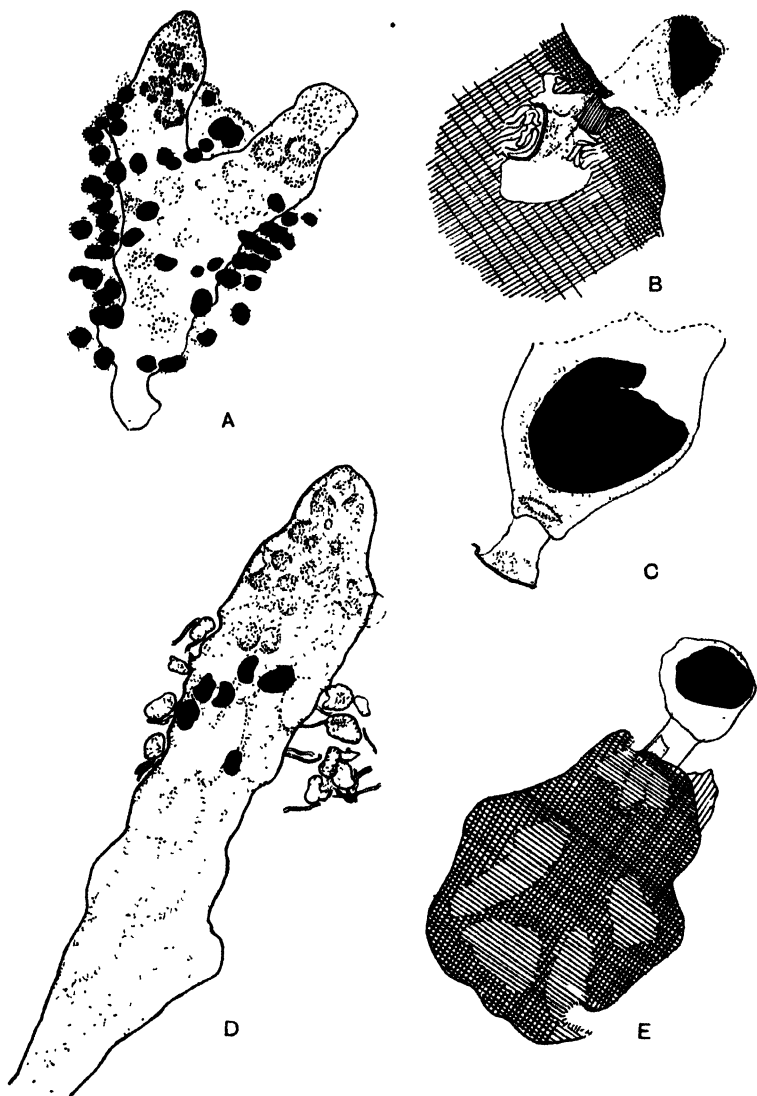


Fig 6. A, *Sargassum lendigerum* Turn. Ktz. Dried material from Umkomaas, Natal, partly cleared, showing oogonia. (Obj 1 inch, eyepiece 5.) B, *Sargassum lendigerum* Turn. Ktz. Part of transverse section of receptacle, showing mucilaginous stalks protruding through mouth of conceptacle. Mucilage partly disintegrated by swelling with lactic phenol. C, *Sargassum lendigerum* Turn. Ktz. One of several oogonia dissected off a receptacle, showing the short stout stalk ending in a disc, probably torn from the exochiton. D, *Cystophyllum muricatum* Turn. J. Ag. Receptacle showing sporelings still in position. From herbarium material (Kew) soaked out and partly cleared. E, *Cystophyllum muricatum* Turn. J. Ag. Diagram of thick transverse section of a younger receptacle, showing the oogonium attached to the interior of the conceptacle. Herbarium material Kew, from Dunk Island, Tropical Queensland, swollen in Tidman's sea salt. (Ocular 2, objective $\frac{1}{2}$.) (Drawings by Mrs F. Laing.)

(b) *Sargassum incisifolium* Turn. J. Ag.

Similar stages in oogonial emission were found in *S. incisifolium*, a South African species, closely allied to *S. lendigerum*.

(c) *Sargassum hemiphyllum* Turn. J. Ag.

The only fertile specimen showing signs of oogonial attachments was a specimen collected by Dr V. M. Grubb in Japan, where the receptacles were surrounded by sporelings, some of which were still hanging by mucilaginous threads.

These three species appear to be unisexual and dioecious so far as can be judged from rather limited material.

V. OOGONIAL RELEASE IN *CYSTOPHYLLUM*

Out of many specimens of *Cystophyllum* examined, oogonial attachments were detected only in a few specimens of the nearly

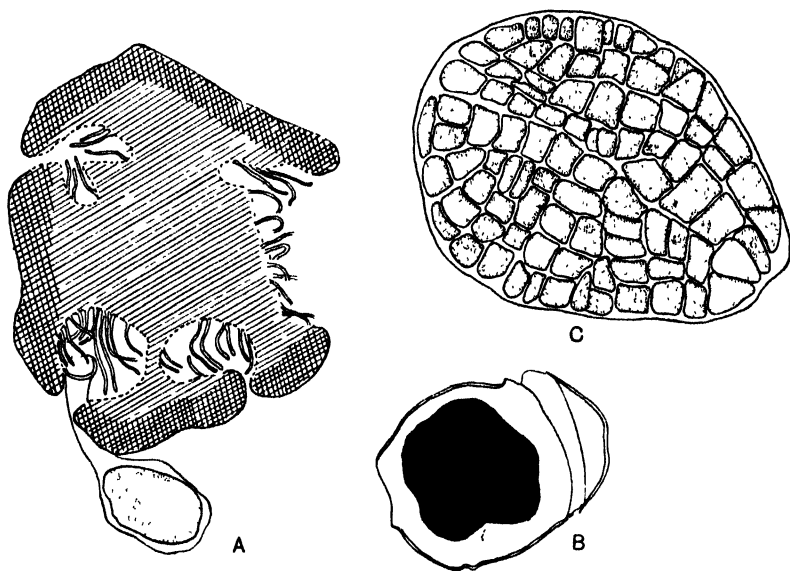


Fig. 7. A, *Cystophyllum trinodis* Forsk. Diagram of thick transverse section through a receptacle showing an oogonium with attachment ending in a refractive disc apparently adhering to paraphyses (the cells of which are not shown). (Zeiss AA, 20 eyepiece.) B, *Cystophyllum trinodis*. Diagram of oogonium nearly ready for extrusion, which after soaking out and mounting cracked with accidental pressure from the coverglass, presumably along the line of dehiscence of the exochiton. C, *Cystophyllum muricatum* Turn J. Ag. Sporeling from receptacle of Fig. 6 D. (Zeiss AA, 20 eyepiece.) (Drawings by Mrs F. Laing.)

allied *C. muricatum* and *C. trinodis*, from the collection at the Herbarium, Royal Gardens, Kew. Samples of the minute receptacles from a number of likely specimens were generously provided by the authorities, and also male receptacles for comparison. Fig. 6 D is a drawing of an entire receptacle of *C. muricatum* softened and mounted after long clearing in cedar-wood oil; this was surrounded in the middle region by sporelings (one of which is shown in Fig. 7 C); above are immature and below are old empty conceptacles. Fig. 6 E illustrates a thick transverse section through a younger conceptacle to which extruded oogonia are attached. A similar section of *C. trinodis* is given in Fig. 7 A, where the stout stalk of attachment ends in a refractive disc as in *Sargassum*. In another receptacle of this species, where the oogonia had not been ejected, an oogonium was seen bearing a cap of mucilage which under slight pressure burst, showing a stout mucilaginous strand emerging (Fig. 7).

The material was unisexual and probably dioecious, though there is no external difference between male and female receptacles, and in the interests of the specimens it is obviously impossible to be sure that the two sexes are only found on the separate individuals. *C. muricatum* is an Australian species occurring in sheltered positions, rarely, if ever, exposed, but subject to rise in temperature when left in shallow pools at low water (Tandy, 1930).

VI. OOGONIA OF *MARGINARIA*

Marginaria boryana Mont. is a gregarious alga of considerable dimensions recorded from deep water off the shores of New Zealand. Amongst several herbarium specimens examined, some bore only male conceptacles on slender cylindrical structures, others only female, on stouter, shorter receptacles; the species is thus presumably dioecious. One of the specimens from the British Museum (Natural History) had female receptacles with whitish hair-like threads protruding from some of the osteoles. A few of these were available for examination: in most the threads proved to be tags of superficial tissue, the material presumably having begun to disintegrate at the surface before drying, but on sectioning one of these, the hairs proved to be the remains of cylindrical mucilaginous strands embedded in abundant structureless jelly, continuous with similar jelly at and within the mouth of the conceptacle. Many of the latter were empty, but some had the remains of old exochitons as more or less complete cups, from a few of which broken strands of mucilage protruded (Fig. 8 D). Occasionally oogonia were found undischarged, and these

were of especial interest, in that the oogonial wall above the solitary oosphere had clear differentiation and was also thicker than that lower down, recalling the "cap" of mucilage described by Kunieda for *Sargassum* and the mucilage of the upper part of the oogonial wall in *Bifurcaria*. In *Marginaria*, the differentiation was most clearly

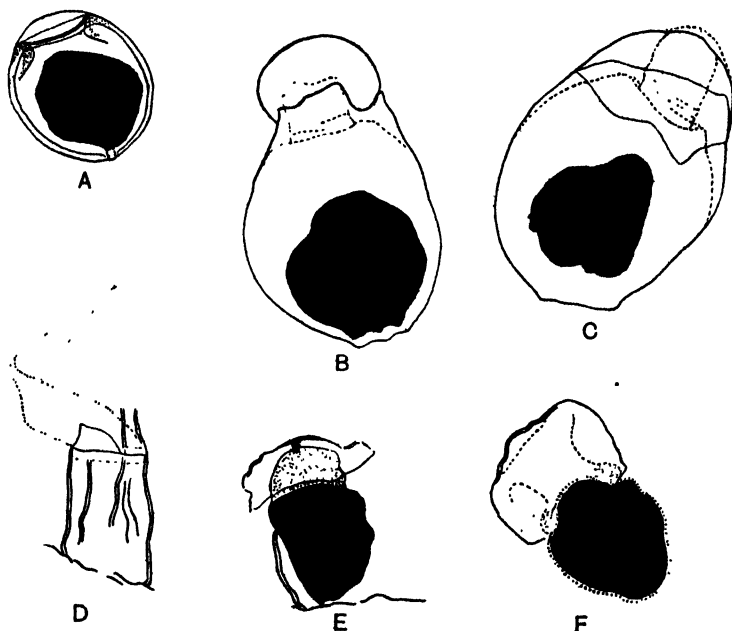


Fig. 8 Oogonia of *Marginaria*. Whole oogonia from nearly ripe conceptacles of *Marginaria*. The part of the oogonium facing towards the cavity of the conceptacle is directed upwards in each case. A, *Marginaria Boryana* Rich. Ripe or nearly ripe oogonium showing "cap" of mucilage with differentiation suggesting an inner denser region B, C, *Marginaria Boryana* Rich. Ripe oogonium, after slight pressure, showing extension of a mucilaginous stalk pushing off a cap of exochiton. D, *Marginaria Boryana* Rich. Empty exochiton with a projecting mucilaginous stalk, from the base of an old conceptacle. E, F, *Marginaria Urvilleana* Rich. Two ripe oogonia in which slight pressure caused the mucilaginous "cap" to extend into a short thick stalk. (Drawings by Mrs F. Laing)

seen when after slight pressure the exochiton burst near the top and a gelatinous band extended slowly at the top of the oosphere (Fig. 8 A, B, C). In the light of these observations, it can hardly be doubted that in this genus also the oogonia are attached for a time after extrusion from the conceptacle. The same appearance of differentiation was found also in one specimen of the only other

species, *M. Urvilleana* Rich., of similar habit. No material has yet been found, however, showing the position immediately after extrusion. Further observations on these plants are in progress.

VII. GENERAL CONSIDERATIONS

The production of secondary attachments to the oogonia is a rare phenomenon limited—so far as at present known—to a few species of *Sargassum* and *Cystophyllum* together with one species of *Bifurcaria*; a similar arrangement probably occurs in *Marginaria*, the two last being of very limited distribution. It is noticeable that all these species occur in relatively warm water, some of them in definitely sheltered positions. Thus *Bifurcaria brassiciformis* occurs most abundantly in Kalk Bay, the water there being about 20° C. warmer than on the more exposed western side of the Cape Peninsula. *Sargassum enerve*, *S. Horneri* and *Cystophyllum sisymbrioides* occur mainly on the coast of Japan warmed by the Gulf Stream. These Japanese species had a definite periodicity. Some such periodicity seems probable in *Bifurcaria*, *Marginaria* spp., also in the species of *Cystophyllum* and *Sargassum*, and would account for the rarity with which stages showing extrusion are to be met in fertile herbarium material.

In addition, these species have a number of characters in common:

(1) They are inhabitants of deep water or of pools near low water of spring tides; thus are permanently, or almost permanently submerged.

(2) They are gregarious in habit, with unisexual, dioecious¹ conceptacles discharging their contents in acropetal succession.

(3) The mucilaginous stalks appear always to form from some part of the inner oogonial wall—apparently the mesochiton—one end being retained within the conceptacle.

(4) The oospheres are solitary and always remain enclosed within a thick gelatinous covering which is continuous with the gelatinous stalk, and probably includes both mesochiton and endochiton.

(5) The oospore germinates within the common jelly which persists for a few days. By this time the germings are multicellular and have formed at least the first rudiments of the rhizoids (Figs. 1, 2). Details of development of the sporelings are known only for *Sargassum* and *Cystophyllum*, and it appears that the rhizoid cell divides before elongating, so that a tuft of short rhizoids is formed at the base of the nearly spherical multicellular sporeling (Fig. 7 C). This is

¹ The dioecious condition is not rigidly maintained (cf. p. 250).

in striking contrast to the mode of germination in *Fucus*, where the rhizoid cell¹ lengthens and becomes a multicellular filament before others appear (Nienburg, 1931).

Kniep believed that moving water was favourable to fertilisation in dioecious intertidal types like *Fucus*, and that submerged types growing in comparatively still water were bisexual and must effect fertilisation almost immediately after extrusion, or the heavy oospheres would sink beyond range of the spermatozooids. This implies that self-fertilisation would be predominant, and to this Kniep attributed the often isolated occurrence of deep-water fucoids such as *Halidrys*.

The unisexual (sometimes dioecious) fucoids of deep water which have adherent oogonia appear to facilitate cross-fertilisation by holding their oogonia in position, so that the spermatozooids released in the neighbourhood have more chance of reaching and penetrating the oogonial jelly. The chemotactic effect of any substance diffusing from groups of oospheres held in position in calm water seems much more likely to be effective than from solitary oospheres tossed by wave action. In *Sargassum* and *Cystophyllum* there is also well-defined periodicity associated with great reduction, the numbers of oogonia in the former being about eight and on the latter only two to three per conceptacle.

A number of submerged types with bisexual conceptacles have also been examined. In these there was no sign of differentiation suggestive of oogonial attachment.

It would be of interest to know whether the distinction between unisexual and (possibly dioecious) and bisexual (monoecious) types of deep water can be maintained in the light of further investigations.

Conceptacles unisexual, with oogonial contents attached; mostly dioecious

Sargassum Horneri Ag.
S. enerve Ag.
S. lendigerum Turn. Ktz.
S. incisifolium Turn. J. Ag
S. hemiphyllum Turn. Ag
Cystophyllum sisymbrioides Mert. J. Ag
C. muricatum Turn. J. Ag
C. trinodis Forsk.
Bifurcaria brassiciformis Ktz.
Marginaria Boryana Rich.
M. Urvilleana Rich.

Conceptacles bisexual, with oogonial contents free

Cystophora uvifera Ag.
Cystoseira ericoides (L.) J. Ag
C. crinita Desf. Bory.
Fucus constrictus Harv.
Bifurcaria tuberculata Stack.
Carpoglossum confluens Ktz.
Scaberia Agardhi Grev

¹ This type of germination occurs also in the enclosed oospore of *Pelvetia*, and was observed by Kniep in sporelings of *Fucus vesiculosus* when the oospore had failed to escape from the mesochiton. However, germination is similar in *Himantalia* and *Cystoseira* where the oospheres are released and spherical germination appears to be correlated mainly with the solitary oospore whether free or enclosed.

VIII. SUMMARY

In the Fucaceae, the contents of the oogonium are released intact; in most species these contents are unattached, and the oospheres are set free by solution of the mucilage.

In some unisexual types with solitary oospheres, the oogonial mucilage forms a stalk, the free end of which is held within the conceptacle while the other end withdraws the egg and pushes it through the osteole. The oosphere remains enclosed and germination takes place *in situ*. This has been demonstrated for three additional species of *Sargassum*, two of *Cystophyllum*, and for another genus *Bifurcaria*; it is probable also in the genus *Marginaria*.

In six other genera with bisexual conceptacles, examined for comparison, there is no sign of mucilaginous attachment. It is suggested that the attachment in unisexual types is a means for facilitating cross-fertilisation in the deep water of the sheltered positions in which these plants are found

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REVIEWS

Paläohistologie der Pflanze. By Dr ELISE HOFMANN. 9½ × 6 in.

Pp. 308, with 153 illustrations. Julius Springer, Vienna, 1934.

"Palaeohistology" sounds recondite and might suggest detailed discussion of the effects of bad preservation on cell membranes; this book, however, is more what I would have called the Anatomy of Fossil Plants. It deals with the more or less fine structure seen in sections and with the cuticle; the whole plant kingdom as represented by fossils is brought under review in some 200 pages and the matter is arranged on systematic lines. The wealth of material has led to selection and the most recent studies of a group are generally taken, though the relevant literature of the last twenty years is mentioned. There is no description of the external appearance of fossil plants, and therefore this book cannot form a text-book by itself, but it forms a useful supplement to other text-books, bringing a large part of the matter up to date. It might also prove valuable by suggesting to teachers of palaeobotany in this country that it is both possible and interesting to give students practical work on fossil plants outside the limits of a collection of Palaeozoic sections.

T. M. HARRIS.

Pflanzensoziologische und bodenkundliche Untersuchung des Schoenetum nigricantis im nordostschweizerischen Mittellande. By L. ZOBRIST.

(Beiträge zur geobotanischen Landesaufnahme der Schweiz, Heft 18.) Pp. 144 with 6 plates. Hans Huber, Bern, 1935. Fr. 9.50.

This book deals with the communities of a particular hydrosere fragment found in the Swiss Mittelland. These communities are said to stand in seral relation to one another in the following way: *Mariscetum serrati* → *Schoenetum nigricantis* → *Molinietum coeruleae*. The middle stage is recognised as divisible into three stages also: *Schoenetum eleocharetosum pauciflorae* → *Schoenetum nigricantis typicum* → *Schoenetum schoenetosum ferruginei*. These communities are described according to the system of Braun-Blanquet.

The book concerns itself chiefly, however, with an attempt to throw light on the causal factors determining the succession. It has involved careful measurement of many edaphic factors in each community, especially acidity, buffering, carbonate and humus content, pore space, air capacity and the content of many mineral elements separately considered. Variation curves are given for the most numerous of these measurements, though their interpretation leaves room for doubt. This is especially so for the pH estimations, for which the smoothed variation curves show very wide overlap between different communities, and maxima which one feels could well have been displaced by a different system of sampling (see Emmett and Ashby, *Ann. Bot.* 48, 1934). It is concluded that the highest carbonate content is in the *Mariscetum serrati*, that humus content and nitrogen reach a maximum in the *Schoenetum ferruginei*, and that the mineral components other than calcium increase gradually in amount through the series to the *Molinietum coeruleae*.

The author concludes that these features are explained by the progressive building up of humus especially in the *Schoenetum*, with corresponding leaching of carbonate and increase of mineral content; the lowered humus content in the *Molinietum* is attributed to the "destructive action" of *Molinia coerulea*.

Although this forms a simple coherent picture, the reviewer feels that there are aspects of the problem which have been somewhat neglected.

Fuller evidence might have been given that the communities here dealt with do stand in successional relation to one another, and not merely a zonal, as may well be the case to judge from the site descriptions and soil analyses. Although the author comments on the increasing dryness through the sequence and even gives a diagram showing the communities in belts on a sloping land surface at the water edge, he makes only incidental measurements of the soil-level, water-level relationship which one would have thought fundamental to a recognition of the mechanism of development of a hydrosere. Nor is the nature of the process of raising of ground level considered more closely, so that silting or accumulation of organic matter or dust might be recognised as a major operating factor. Furthermore, one may complain that anthropogenous factors, though mentioned, are quite inadequately considered in their successional implications: we are told that the *Schoenetus nigricans* and *Molinietum* are cut for litter and that one sample of the *Mariscetum* community is mowed, in all of these seedling bushes of *Frangula alnus*, *Salix cinerea*, and *Fraxinus excelsior* appear and yet it is not made clear whether or no effective bush invasion of uncut sedge can take place. If it can do so as in some English fens, then the succession here dealt with is possibly dependent on grazing, cutting, absence of seed parents of bushes and trees, or upon some circumstance of development not yet apparent but equally serving to deflect the succession from the simple sequence *Mariscetum serrati* - fen scrub.

These criticisms will serve still further to point out the difficulties of elucidating the most fascinating of ecological studies, the mechanism of the operation of vegetational change.

H. GODWIN.

Flora von Graubünden. By J. BRAUN-BLANQUET and E. RÜBEL. Dritte Lieferung. (Heft 7 der Veröffentlichungen des Geobotanischen Institutes Rübel in Zürich.) Pp. 380. Hans Huber, Bern und Berlin, 1934. Fr. 22.50.

The first and second volumes of this flora of south-eastern Switzerland have already been reviewed in this journal; the third carries on the same treatment from the Leguminosae to the Solanaceae. It is of great interest to the ecologist to compare the habitat and distribution data given here with those for the same species in other parts of Europe. *Ilex aquifolium*, which reproduces readily in Britain, will be found in the *Graubünden* to be limited to very moist regions with small extremes of temperature, it is injured by frost and does not then produce fruit. *Rhamnus catharticus* is not indicated as showing the preference for calcareous soil which it has in this country, nor is *Frangula alnus* indicated as specially common on wet sandy acid soils, but "sowohl auf kalkreichen als auf völlig kalkfreiem Boden". The latter species is mentioned with *Salix cinerea* as one of the earliest pioneer bushes invading bogs, just as in the fens of Cambridgeshire and the Norfolk Broad. *Hippophae rhamnoides* (which, incidentally, has been omitted from the *Index generum*) is shown in its well-known alpine role as early colonist of sandy alluvium along mountain streams, where it is a most important stabilising influence, as also on the damp soils of steep mountain sides. In this country, where the shrub is now limited to the coastal sand dunes, this should serve to remind us that *Hippophae rhamnoides* may well have been widespread in the period closely following retreat of the ice at the end of the glacial period. Apart from the distributional data which it is the first object of a flora such as this to provide, the points we have mentioned will serve to show the wider interest of this particular work.

H. GODWIN.

Elementary Microtechnique. By H. A. PEACOCK, M.Sc. $7\frac{1}{2} \times 5$ in.
Pp. viii + 200. Edward Arnold, 1935. Price 5s. 6d.

During the time that he has been in charge of the biology department at Cheltenham Grammar School, Mr Peacock has assiduously collected and tested a very wide range of laboratory processes concerned with the preparation of plant and animal material for microscopic examination by senior school students and first-year university men. These are now published in a very useful and inexpensive volume, which succeeds admirably in the author's intention of filling the gap which exists between too brief laboratory instruction sheets and exhaustive and expensive text-books.

The author rightly lays stress on the principles which underlie the processes of microtechnique, and gives very full instructions in all fields of this study. It is pleasant to find careful instructions on the care and sharpening of the razor and on the use of the microscope. There are three appendices on sources and culture of material, on preservation of material, and a short bibliography of advanced texts. The preparation of stains, mounting and culture fluids and so forth are given with full detail in the text.

All laboratory workers will have felt at some time or other the need for some such reference book as this, and will thank the author for his labour of collection and organisation. We may hope also that they will assist him by suggestions for improvement, for it is inevitable that many excellent pieces of elementary microtechnique have still been omitted. Especially on the plant side we would suggest that a most useful method of examining fungal mycelium within the host tissue is by staining in hot lacto-phenol and mounting in phenol. We should also like to protest against the lack of distinction between specific tests for lignin (such as the phloroglucinol and aniline chloride tests) and cellulose (such as iodine in zinc chloride), and the general results of differential staining with two dyes, either of which is capable of imparting colour to all the tissues of a section and which operate together by differential displacement to give a final distinction between lignin and cellulose. The author may be referred to the note on this subject contributed to a recent number of this journal by Prof. MacLean.

In conclusion the reviewer may be allowed to express the hope that this book will not lead any school student to attempt more than a fraction of the technique here described, the capacity for making full use of unstained sections, and in fact the direct observation of living material are the real foundations of biological training rather than the practice of elaborate and specialised microtechniques.

H. GODWIN

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A PRELIMINARY STUDY OF SOME SNOWDONIAN PEATS¹

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(With 11 figures in the text)

INTRODUCTION

THE Snowdonian peats described below lie mostly in the Nant Ffrancon (Fig. 1), a mountain pass drained by the River Ogwen and traversed by Telford's great London-to-Holyhead coach road. The principal records are based on samples taken from the higher reaches of the pass and especially in a lateral corrie (Cwm Idwal), once occupied by a hanging glacier that overrode the main Ogwen glacier occupying the bed of the Nant Ffrancon. The watershed is approximately 1000 ft. above sea-level, and the mountain streams flow into a shallow lake, Llyn Ogwen, through peat areas: Jehu (7) envisaged the probability that Llyn Ogwen occupies a basin eroded by glacial action "during a late stage of the Glacial Epoch, when the ice had retreated from the lower reaches of the valley". At the foot of this lake the river receives the stream from Cwm Idwal, at the confluence pouring over a fine cascade (Rhaiadr y Benglog) into the main valley that has a north-south trend. The valley has a flat floor. The hanging valleys on the west flank (Cwm Cywion and Cwm Perfedd in addition to Cwm Idwal and Cwm Bochlywd) have steep walls rising to an altitude of 3000 ft.: the eastern flank is a steep lofty shoulder of the Carnedd group of peaks. Some of these cwms (corries) contain small tarns, around the margins of which peat deposits have been sampled.

¹ This paper embodies part of the material presented by L. M. Hodgson (6) for the degree of M Sc. in the University of Wales

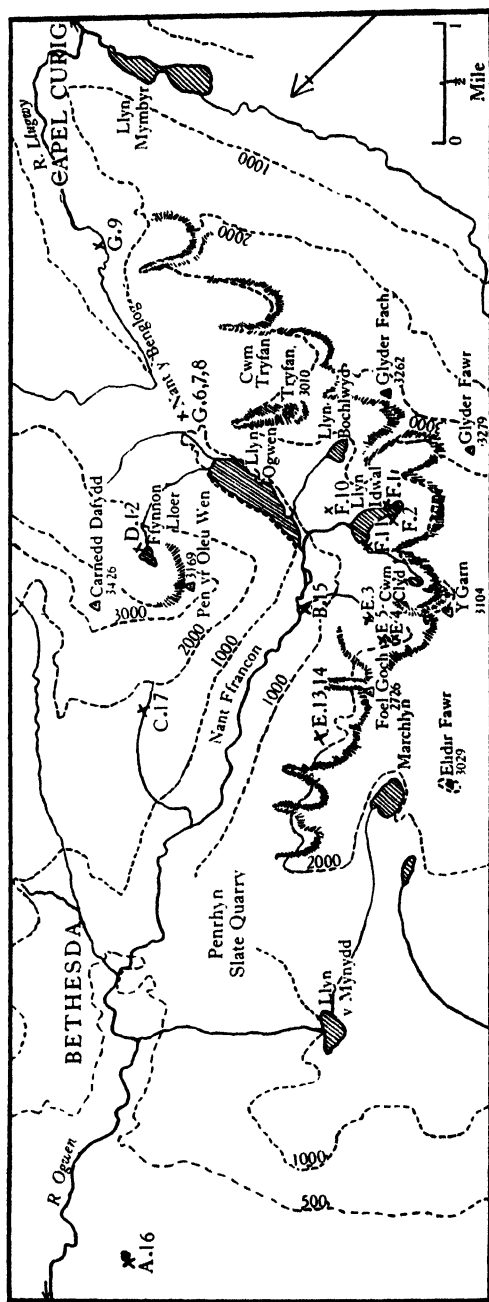


Fig. 1. Sketch-map of the Nant Francon, Caernarvonshire, showing the position of the peat deposits.
Heights of contours and peaks in feet above sea-level.

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In all seventeen spots were examined in the years 1931-2; they are indicated in the sketch-map by topographical letters together with the field numbers, and are listed in Table I.

TABLE I

Peat No	Situation	Altitude ft.	Maximum depth recorded ft.	Remarks
A 16	Cororion	250	7	Recent peat, lake silting up. Reed-swamp surrounded by willows and alders
B 15	Blaen y Nant	500	8	Extensive peat on valley floor at head of valley. No evidence of peat cutting
C 17	Ty'n y Maes	1900	5	Peat cutting doubtful
D 12	Ffynnon Lloer	2200	4	Small pocket on windward side of valley
E 3	Cwm Cywion, below the tarn	1550	2	Peat forming at present
E 4	Cwm Cywion, south of the tarn	1750	7	Two parts of same deposit. The tarn is almost silted up
E 5	Cwm Cywion, north of the tarn	1750	1	
E 13	Cwm Perfedd }	1750	1.8	Small pocket on leeward side of valley
E 14	F 1 }			
F 1	Cwm Idwal, east of Llyn Idwal	1300	2	Only the superficial layer examined. Much eroded
F 2	Cwm Idwal, west of Llyn Idwal	1250	14	Between moraines of retreat. Erosion quite recent and only partial
F 10	Ogwen Cottage	1200	3	No erosion; covered with <i>Juncetum squarrosus</i>
F 11	Cwm Idwal, stream from Cwm Clyd	1200	1	Between clay below and overlying water-worn detritus
G 6	Gwern y Gof, 1.8 miles from Ogwen Cottage	1000	4	Peat is cut in these areas at the present time
G 9	Gwern y Gof, 3.3 miles from Ogwen Cottage	800	6	Do.

Within a few square miles this series of profiles includes a wide range of altitude from Cororion, at the foot of the valley, to highland peats at about 2000 ft. The latter lie within the limits of glaciation as determined geologically. The aspect and area of the spots also show a wide range. Most of the peats are covered with closed moorland associations, but others are partly eroded or worn down to such an extent that the base of the peat is exposed in patches, or rock is reached by probing a foot or so from the surface.

For many decades, extensive peat-cutting and turbary rights have been held by individuals and parishes in Wales, the Enclosure Acts reserving a share of the turbary for the commoners. We are indebted to Dr R. Alun Roberts for the information that a close study of the

history of rural Wales, and of the mountain grazing lands of the district, make it probable that the majority of the Nant Ffrancon peats were untouched, except, perhaps, the Ty'n y Maes peat (C 17). The peats in Nant Benglog (group G), between Llyn Ogwen and the village of Capel Curig, are cut for fuel at the present day, but the remainder are undisturbed.

METHODS

Most of the samples were obtained after cutting a trench through the peat to its base, cleaning one of the vertical faces with a large knife, and either slicing a column from top to bottom, or taking samples from the freshly exposed trench wall. This trenching method gave also valuable information regarding the bulkier plant remains. In the deepest profiles or when the trenches reached the water-table, borings from the base with a simple type of soil auger supplemented the higher samples.

As it was thought of interest to obtain some estimate of the relative abundance of total pollen at different horizons, 2 gm. of moist peat were taken as the unit for extraction. The technique cited by the Erdtmans(4) in 1933 was not then published. Several methods of extraction were tried. Potash proved more efficient than soda in clearing away the plant debris. The usual reagent was 10 per cent. KOH, and peats were boiled for as long as 6 hours. In comparison with samples rapidly boiled up on a slide, no appreciable disintegration of the grains was noticed. Most peats were sufficiently cleared for microscopical examination after 2 hours' simmering in alkali.

The treated peat was added to a glycerine jelly of a very low water content (glycerine 70 c.c., water 10 c.c., gelatine 15 gm., and a few crystals of phenol). Gentle heating in a water bath rendered this "peat-glycerine-jelly" liquid enough to be pipetted on to slides by means of capillary pipettes of known volume. The pipettes were subjected to careful cleansing in boiling water after delivery of their contents, and as a further precaution to prevent pollen from one sample being carried over they were stored in methylene blue. Any pollen left behind would be stained when the next sample was discharged. In practice it was found that the capillary pipettes were thoroughly cleaned by the boiling water. Staining was unnecessary; the acid nigrosin recommended by Moore and La Garde(9) for fresh pollen from herbaceous plants failed to stain the peat pollens. The mounts were usually sealed with Canada balsam or hyrax.

Other permanent preparations were made by washing the pollen

grains, after alkali treatment, in alcohol. The alcohol suspension was pipetted on to a clean coverslip and, when the spirit had evaporated, the coverslip was inverted into a little glycerine jelly on a slide. The method gave satisfaction and the grains were well preserved. Canada balsam was not a satisfactory mounting medium.

Sufficient pollen was counted in each analysis to include at least a hundred tree pollen grains. Identification from Meinke's *Atlas* (8) was supplemented by examining fresh pollen, especially of trees. A number of pollen grains yet await exact identification.

Among the pollen grains occur spores of mosses and ferns, principally of *Sphagnum* and *Polytrichum* with *Pteridium* in the lower horizons.

The coarser plant remains were separated from the peat by short treatment with hot 10 per cent. KOH and straining. After washing, most of the material could readily be teased for microscopical examination. The wood fragments were hand sectioned or macerated with Schultze's macerating mixture. The timbers were compared with Stone's descriptions (10), and bark specimens with fresh material.

ESTIMATION OF SAMPLING ERRORS

There are so many factors complicating the interpretation of absolute pollen frequencies from sample to sample, or from profile to profile, that it is extremely difficult to find a basis on which such an analysis can be usefully employed. These limitations have been discussed recently by Godwin (5), who, however, points out that "the method will always indicate changes in the *relative* abundance of the pollen of the various tree genera". In this account only the measurements of the relative pollen frequencies are dealt with, except in so far as the ratio of the total tree pollen to total pollen yields data to supplement evidence obtained in other ways.

When the work was done, no statistical treatment of the errors involved in the technique of pollen analysis was found in the literature available to us, but it seemed obviously desirable that some estimate should be obtained of our own sampling errors. Three steps may be distinguished:

(a) The error in the determination of the relative pollen frequencies in 2 gm. of moist peat.

(b) The sampling error in taking 2 gm. from a larger sample.

(c) The sampling error in taking a boring at one point in a peat area.

Figures required were obtained from the Blaen y Nant peat

(B 15) which was chosen on account of its accessibility. The results are given in Table II: 150 grains were counted in each analysis and only the data for the major constituents are included in the table. As it is often necessary to make a number of preparations to get enough tree pollen, ericaceous pollens (noted as *Erica*) have been counted in with the tree pollens in order to obtain, without undue labour, figures for higher and lower percentages.

TABLE II

	*	1	2	3	4	5	6	7	8	9	10	Mean	Standard deviation	Standard deviation of mean
(a) Analyses of ten separate mounts from the same 2 gm. preparation.														
<i>Erica</i>	77	77	75	77	67	72	75	72	75	75	72	73.7	± 2.8	± 0.9
<i>Alnus</i>	4	4	7	7	8	7	7	5	6	3	8	6.2	1.6	0.5
<i>Corylus</i>	8	8	5	5	11	5	5	8	7	6	8	6.8	1.8	0.5
<i>Betula</i>	8	8	14	10	15	16	14	15	13	16	12	13.3	0.4	0.8
(b) Analyses of ten different 2 gm. preparations of the same peat sample.														
<i>Erica</i>	77	77	68	66	72	77	77	77	80	80	69	74.3	4.8	1.5
<i>Alnus</i>	4	8	8	13	5	5	7	5	4	6	5	6.1	2.5	0.8
<i>Corylus</i>	8	4	9	6	9	7	5	5	5	3	7	6.0	1.9	0.6
<i>Betula</i>	8	8	14	17	14	10	11	13	11	11	19	12.8	3.1	1.0
(c) Analyses of ten samples taken from the uppermost layer of the peat from places up to 60 ft. apart.														
<i>Erica</i>	77	77	69	80	72	80	80	67	77	69	72	74.3	4.8	1.5
<i>Alnus</i>	4	4	7	5	6	5	5	9	5	9	8	6.3	1.7	0.5
<i>Corylus</i>	8	8	5	6	6	4	5	5	8	11	7	6.5	1.9	0.6
<i>Betula</i>	8	8	19	17	15	11	10	19	10	11	13	12.9	3.5	1.1

* The first column gives the figures previously obtained for this horizon.

The third section of Table II must inevitably include the sampling errors inseparable from those in the second section, which in turn includes the sampling errors of the first section.

RESULTS

In dealing with the results of the examination of the seventeen peat profiles, number F 2 will be considered first in detail as this has given the longest and most complete sequence. Others will then be compared with this.

The Cwm Idwal deposit (Figs. 2, 3)

The principal peat deposit in Cwm Idwal which provided profile F 2 occurs between grassy mounds lying parallel to and above the western shore of Llyn Idwal. They are probably moraines of retreat (Jehu(7)). The cwm has been fully described by Sir Andrew Ramsay in his classical work, *The Old Glaciers of Switzerland and North Wales*.

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It is appropriate that the centre of Ramsay's observations on Welsh glaciology should yield the most complete record of post-Pleistocene peat deposition.

This locality has been kept under observation since 1926, when a quadrat was established on the bog. The peat is being eroded by

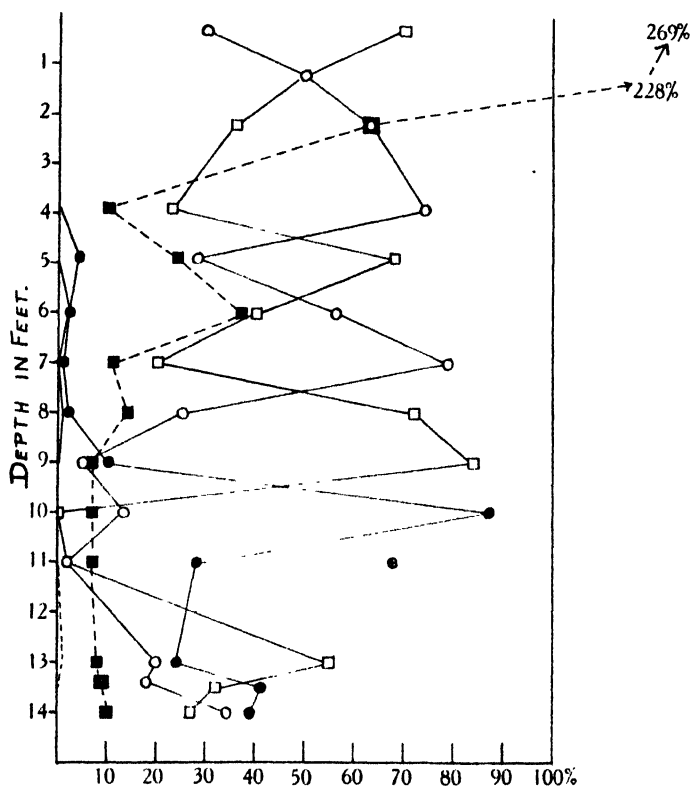


Fig. 2. Pollen diagram of peat profile F 2, Cwm Idwal.

Alnus —□— *Betula* —○— *Corylus* —■— *Pinus* —●—
Quercus — — — *Salix* ⊕ *Tilia* — — —

water draining from Y Garn above, forming a shallow stream across the area, and having three outlets. The hags project 2 ft. above the surface of the exposed peat. The brown surface is covered with plant remains and partially embedded stools of small trees. "Soundings" through the peat at various points showed depths from 4 to 14 ft.

In digging the trench the water-table was reached at about 10 ft. A study of the strata revealed a contrast between

(a) An upper zone, 5 ft. deep, where ericaceous pollens outnumber the tree pollens.

(b) A lower zone, 9 ft. deep, where tree pollen predominates to the ultimate exclusion of heath.

In the upper zone, *Alnus* and *Betula* are alone represented among forest pollens; their frequency is so small that a large number of

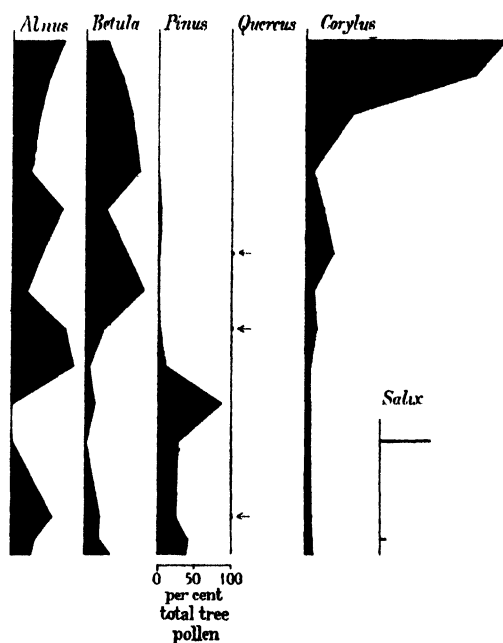


Fig. 3. Profile F 2; Cwm Idwal. Dissection of the pollen diagram shown in Fig. 2.

mounts have to be made in order to obtain a satisfactory count. (Thus in the sub-surface sample over a thousand grains, mostly ericoid, were counted.) Not only does *Alnus* decrease as *Betula* increases to a maximum at 4 ft. deep, but also there is a marked decrease of other pollens, until *Betula* accounts for a third of the total pollen. Down to this level the macroscopic remains are mainly birch twigs, bark and small stumps, the latter mostly embedded in the hags. Their size indicates that the trees were small. In Meinke's *Atlas* (8) no difference is noted between the pollen grains of *Betula*

pubescens and *B. alba*, but the Idwal remains have the timber characters of the former species. At the next horizon, 5 ft. deep, alder wood specimens are found along with hazel nuts together with the birch. This horizon marks the last appearance of *Pinus* pollen in the peat. *Alnus* has a sharply defined maximum over *Betula* at this level.

The lower zone has several well-defined maxima. At 6 and 7 ft. levels, *Betula* again establishes a maximum, which, when expressed in terms of total pollen, reaches the high proportion of 70 per cent. *Alnus* is the next most abundant pollen, *Pinus* and *Quercus* having very subordinate positions. *Corylus* has also only a small representation. No specimens of birch wood are found in this or deeper layers, however, and the alder specimens at the 6-ft. level are large. It is noteworthy that the most abundant remains of these two trees occur at levels immediately above their pollen maxima.

Birch only occupies a subordinate position in all the deeper horizons. At 9 ft. *Alnus* shows a maximum, while the pollens of other identified woody plants remain approximately unaltered and similar to one another in their proportions.

At the 10-ft. level, alder is very rare, but *Pinus* takes its place, showing a higher proportion than any of the previously mentioned maxima. Wood remains of the pine have also been found, one stump in a vertical position, probably *in situ*. No wood remains have been obtained below as trenching gave place to boring. *Salix* is common at 11 ft. with the pine. Near the base of the peat there is a tendency for all the major tree pollens to be present in more or less equal proportions; alder ousts pine at 13 ft., but becomes subordinate to pine and birch at the lowest horizon. Birch shows a steady increase from 11 ft. downwards. In all these deeper layers, and especially below 8 ft., hazel pollen remains at a constant and low percentage (about 10 per cent.).

The other peats in the Nant Ffrancon may be compared with the Idwal peat, F 2, as a standard.

Other Cwm Idwal deposits, F 1, 10, 11 (Fig. 4)

Three other deposits in the Cwm have been examined. A peat, F 1, at the lake head beneath the Idwal Slabs, has been greatly worn down almost to its base. Birch fragments are scattered freely on the bared brownish surface. Only the subsurface sample has been analysed and, in general, its pollen flora agrees with that of the

upper zone of the standard deposit, alder predominating over birch and hazel occurring in abundance. This horizon is identified with the uppermost layers of profile F 2.

F 11 is a narrow strip scarcely more than a foot in thickness, buried under 2-3 ft. of water-worn detritus brought down from the cascade of the stream draining Cwm Clyd. The deposit is exposed in section on the right bank of the stream before it debouches into Llyn Idwal. It is packed with birch twigs. Few pollen types are found in it, but they agree closely in species and relative frequency with those

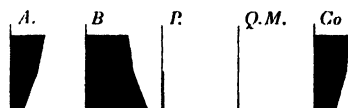


Fig. 4. Profile F 10; Ogwen Cottage.

In Figs. 4-11 each vertical column shows the percentage frequency of each tree species expressed in terms of the total tree pollen. The symbols used are those usually adopted *A.* *Alnus*; *B.* *Betula*; *P.* *Pinus*; *Q.M.* *Quercetum mixtum* (mixed oak), *Co.* *Corylus*; *S.* *Salix*.

of the 2-ft. horizon in the main peat, except for the lower proportion of hazel.

Separated from the main cwm by the terminal moraine lie the Bochlwyd marshes in which is situated the Ogwen Cottage deposit F 10 (Fig. 4). It is 3 ft. deep with birch twigs lying in the lowest part. *Pinus* pollen is present in small amounts except in the topmost sample, *Betula* shows an increase from top to bottom, while *Alnus* and *Corylus* both decrease. There is very little ericoid pollen at the base. The pollen diagram, taken together with the macroscopic remains, suggests a correlation with the horizons between 5 and 6 ft. deep in the main peat. This peat has been undisturbed by erosion.

The Nant y Benglog deposits, G 6, 7, 8, 9 (Figs. 5, 6)

These are separated from the Cwm Idwal group by the rocky mass of Tryfan. They are more extensive and have been subject to sporadic cutting for fuel by the hill farmers, but have never been so severely cut as the turbaries elsewhere in the county. The profiles, G 6, 7 and 8, are from the same deposit at the foot of Cwm Tryfan and at the watershed. The deepest of them, G 6 (Fig. 5), is 4 ft. thick, alder and birch wood being found at the bottom. *Corylus* shows a marked decrease in the uppermost few inches. The configuration of the principal pollen curves is very similar to the upper 5 ft. of the

Idwal standard, with an abundance of alder at the base. Peat formation commenced here after the disappearance of pine.

Nearer Capel Curig, where the valley widens, there is a slightly deeper deposit G 9, (Fig. 6). Just below the surface *Pinus* pollen is found coinciding with an alder maximum, comparable with the 5-ft. level at Idwal. *Corylus* is scanty but increases to the fourth foot where *Quercus* is a minor associate. Alder is also subordinate to the

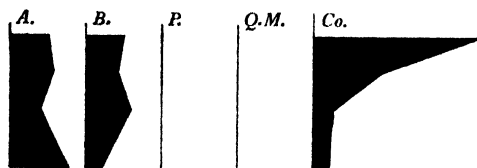


Fig. 5. Profile G 6; Nant y Benglog.

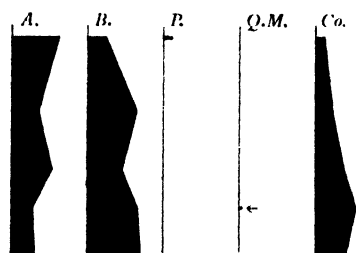


Fig. 6. Profile G 9; Gwern y Gof.

birch-hazel pollens. birch twigs occur in a layer immediately above the horizon where its pollen is abundant. A study of the associated pollens shows that there are several types of pollen grains present both in the lower half of this profile and also in the zone between 4 and 7 ft. in the standard profile. The origin of this profile was synchronous with that of profile F 10, but conditions for peat deposition were more favourable on the Nant y Benglog side of the watershed.

The Ffynnon Lloer deposit, D 12 (Fig. 7)

This lies in a high corrie above Nant y Benglog under the summits of Carnedd Dafydd and Pen yr Oleu Wen. It is the highest of the deposits (2200 ft.) and has a southern aspect. It is only small in extent, lies close to the tarn in the corrie, and has a maximum depth of 4 ft. At each horizon the tree pollen is only a small proportion of the total pollen: the site is over 1000 ft. above the present-day tree

limit. *Corylus* is abundant throughout the profile which mostly shows an *Alnus* maximum. *Pinus* and some *Salix* occur at the base and are replaced above by *Quercus*, which, however, does not persist to the surface. Other pollens compare closely with those found in the

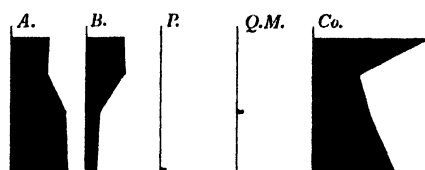


Fig. 7. Profile D 12; Flynnon Lloer.

Idwal horizons between 3 and 6 ft., excepting a greater abundance of ericoid pollens. The abundance of hazel may be due to hazel scrub on the slopes of the Carnedd Dafydd Range, from which pollen may easily have been blown by the prevailing winds up into the hollow.

The Blaen y Nant deposit, B 15 (Fig. 8)

Turning to the peats north of Cwm Idwal, attention is first claimed by the Blaen y Nant deposit on the alluvial valley floor immediately under the lateral wall of Cwm Idwal. It must be

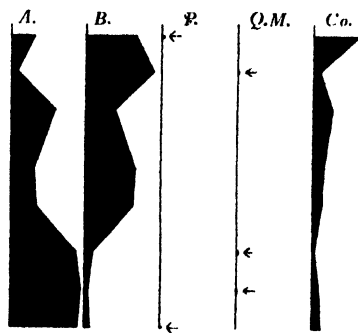


Fig. 8. Profile B 15; Blaen y Nant.

borne in mind that this valley bottom was the bed of a long deep lake when the main glacier retreated. It is difficult to determine how long a time elapsed before it drained away, as the water was not impounded by morainic debris (the glacier snout lying far to the north) but by a rocky wall near Ty'n y Maes. Peat formation would be necessarily delayed until the lake had started to empty, and the

lowering of the water level would permit peat to form at the head of the valley before the lower end.

The deposit is 8 ft. deep; the lowest samples were obtained by boring. Near the surface, birch is the predominant pollen with alder and a little pine: a few oak grains were found at 15 in. The middle section shows an increased percentage of alder, but birch becomes dominant again between 3 and 5 ft. Hazel pollen diminishes. Below 5 ft. to the base of the profile, alder replaces birch and accounts for 80-90 per cent. of the tree pollen. Here pine, oak and lime also occur, the latter only represented by a few grains. Birch fragments are found in the surface 3 ft. and then become mixed with alder down to 5 ft., below which alder wood alone is found: a similar change has been described in the Cwm Idwal deposit (see p. 271). It appears that this deposit began its history contemporaneously with the 9-ft. horizon in Cwm Idwal, and that peat formation was more rapid than in the cwm above, for the pollen curves in the upper section correspond closely with those of the third and fourth feet in the standard profile. During this period a peat 8 ft. in thickness had been laid down against about 6 ft. in the higher deposit.

The Cwm Cywion deposits, E 3, 4, 13, 14 (Fig. 9)

This remote and seldom visited cwm, between the mountains Y Garn and Foel Goch on the west flank of the Nant Ffrancon, once contained a large tarn, now restricted in size to a small bog pool

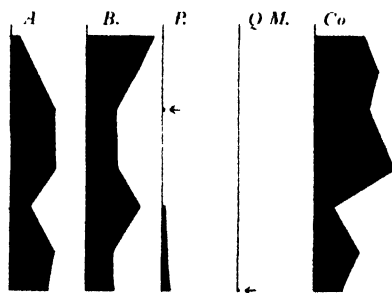


Fig. 9. Profile E 4, Cwm Cywion.

surrounded by extensive peat. The area of peat stretches across the corrie and has not been thoroughly probed. Part of the deposit is eroded, some of the hags standing 5 ft. high: this erosion is balanced by peat formation nearer the tarn.

A small pocket of peat, E 3, about 200 ft. below the corrie has

remains indicative of its formation in late subatlantic times with a high birch pollen frequency. Profile E 4 (Fig. 9), taken at a spot 200 yards from the margin of Llyn Cywion, is a deep peat. From the upper horizon to the third foot there is a decrease in the frequency of birch pollen, which is abundant at the top. At 27 in. pine pollen is present. It occurs again at about 5 ft. and gradually increases downwards. The middle zone has alder replacing birch as the principal tree pollen, hazel being prolific to the fourth foot, below which it decreases. Birch shows a maximum at 5 ft., immediately below which the alder and birch curves cross. Among the macroscopic remains birch wood is found to a depth of 4 ft., but alder wood is found more freely in all layers. At the lowest level lime pollen is present. The pollen curves and wood specimens suggest that the base of the peat was contemporaneous with the 9-ft. horizon in Cwm Idwal and with the Blaen y Nant peat, over 1000 ft. below, which is cut across by the stream draining Cwm Cywion. Seven feet of this profile correspond with 5 ft. of the Idwal deposit which suggests that peat accumulation was more rapid.

Another shallow pocket of peat has been examined in a neighbouring corrie to the north, Cwm Perfedd (E 13 and 14). It belongs to a birch period, alder and pine being present. An unidentified pollen grain of a rosaceous type is present which is only found in certain horizons, corresponding with the 3-7 ft. zone elsewhere in the valley, and in Cwm Idwal, and its occurrence strengthens the assumption that this little patch is to be dated contemporaneous with this period.

The Ty'n y Maes deposit (Fig. 10)

This peat, C 17, is of great size, situated on the more gently sloping northern side of Carnedd Dafydd at a height of 1900 ft.

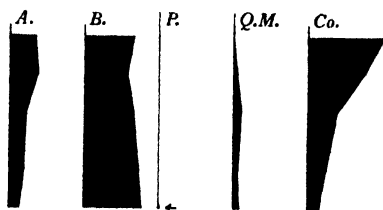


Fig. 10. Profile C 17; Ty'n y Maes.

It is drained by a small stream that falls into the main valley at the hamlet of Ty'n y Maes, i.e. at the narrow constriction that may have held back the waters of the early post-Glacial lake. The peat has

two definite strata where plant remains are closely packed together; the upper stratum is 4-5 in. thick, of birch twigs, lying under 2 ft. of peat. Immediately below this stratum remains are sparse, but in the lowest 2 ft. of the profile there are larger birch trunks in profusion. *Betula* is the predominant tree pollen in all layers. *Corylus* is very common at the top but rapidly becomes subordinate. The mixed oak pollens have a higher relative frequency in this deposit than in any so far considered, and their occurrence is more continuous. Alder is present throughout in moderate proportions, and pine is found at the bottom. The whole of this 5 ft. of peat corresponds with a narrow belt, from 5 to 7 ft., of the standard profile and may illustrate how the degree of peat accumulation depended on local topographical conditions in a limited area.

The Cororion deposit (Fig. 11)

This deposit, A 16, lying in the lower section of the Ogwen valley, is the only one on which there are living trees. A small lake, Llyn Cororion, closely resembles a Cheshire mere, and it is screened on three sides by a thick game cover of *Betula*, *Alnus*, *Salix* and

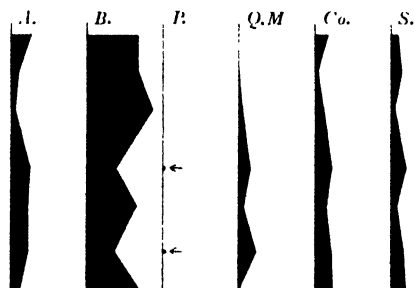


Fig. 11. Profile A 16, Cororion.

Sambucus with *Myrica* and *Pteridium*. An examination of old maps shows that there has been steady encroachment on the open water during the last hundred years by a *Phragmites-Scirpus lacustris* fringe advancing in front of the *Sphagnum*. The profile selected for analysis lies near to the periphery of the deposit, a few yards away from where it gives place to meadow land. It is thus older in origin than the saturated peat nearer the reeds 50-100 yards away. Rough tussocks of *Molinia* and patches of *Polytrichum* cover the peat in this outer zone, which is nowhere eroded. Despite its proximity to the

meadows, the profile is 7 ft. thick, and as the water-table lies close to the surface most of the horizons were obtained by boring.

The pollen of living trees is meagre in the topmost horizon, showing that very little pollen is washed down through the covering of vegetation. All the horizons are characterised by a high percentage of birch; it shows some fluctuations but always maintains a higher proportion than that of the other tree pollens. Just below 2 ft. it accounts for 90 per cent. of the tree pollen (75 per cent. of the total pollen). At this level there are also a few remains of twigs and leaves of the tree. Oak and alder, with some willow, of which two species are probably represented, make up the tree pollen and, with hazel, maintain a constant frequency to the base of the profile. Pine and ash appear in the lowest 3 ft.

The pollen curves for this profile correspond with those of the lower zone at Ty'n y Maes and the zone in Cwm Idwal to which the other profiles have been referred. An examination of pollens, other than forest types, confirms this view and also helps to establish a connection between it and the Blaen y Nant peat at the head of the Nant Ffrancon trough. At least three unidentified grains occur only in these layers. The thickness of the Cororion peat and its homogeneity suggest that conditions were very suitable for its formation during this period. It is possible that a series of borings between this spot and the margin of the existing pool might give a series of overlapping chapters in the story from then to the present day. The profile is so close to the periphery that it is unlikely that borings in the other direction, away from the pool, would push the origin back much earlier than that already determined. The base of the Blaen y Nant deposit is a good deal earlier than the Cororion peat, and the difference between them may have some relation to the date of emptying of the great Nant Ffrancon lake.

General

The matrix of all the Nant Ffrancon peats is *Sphagnum*; moss spores are common in most of the samples, often mixed with fern sporangia and spores. Polypodioid sporangia are commoner in the later deposits than pteroid sporangia, but in the earlier deposits the position is reversed.

Except in its lower reaches the valley is no longer wooded apart from a few small plantations and a birch scrub on the valley floor near Ty'n y Maes, ending abruptly several hundred feet below the nearest deposit examined. The existing tree limit in Caernarvonshire

lies at about 900 ft., but most of the profiles are taken from altitudes above that level. The large fragments of timber found in some of the peats provide evidence that conditions permitted the growth of trees actually on these highland peats at certain periods; they have been found as high as 1900 ft., and this makes it certain that the tree limit once lay 1000 ft. above its present position. It seems probable that when the climate changed and peat accumulation began, the trees were gradually killed off, and some of their trunks partially preserved after embedding in *Sphagnum*, while pollen from trees around the margin or from greater distances continued to blow on to the new surface. The Cororion deposit is in such a transitional stage to-day, most of the trees being in a poor condition and their bases partly buried by *Sphagnum*.

DISCUSSION

Though these profiles show such a wide range of altitude, exposure and aspect, most of them have basal horizons that can be correlated with a comparatively narrow band in the Cwm Idwal profile, the band from 3 to 7 ft. All of the profiles include horizons of this period and most of them lie entirely within it. Only three of the deposits had an earlier origin, and in these the optimum phase is pronounced. The features of these horizons are the low percentage of *Pinus* and mixed-oak pollens (*Quercetum mixtum*), a moderate percentage of *Alnus*, and, generally, a high percentage of *Betula*. Throughout the Nant Ffrancon, peat formation was general at this time: it had commenced earlier in Cwm Idwal itself.

The surface deposits have a tendency towards a pronounced hazel maximum (Figs. 3, 5, 7, 9, 10), for hazel pollen grains outnumber the whole of the generally recognised tree grains. In them *Pinus* is absent and *Quercetum mixtum* either absent or only slightly represented. As *Alnus* and *Betula* are the only tree pollens present their fluctuations may have local ecological interest only, and have no reliability as records of major climatological changes.

Though Caernarvonshire is rich in megalithic and other archaeological remains, yet the Nant Ffrancon and its tributary cwms have no known ancient sites. On the crests of the Carnedd group and elsewhere adjacent to the valley there are tumuli, carneddau, etc., of Neolithic Age. No worked implements have been recorded from any of the peat areas examined: a high peat on Carnedd Llewelyn, as yet unexamined by methods of pollen analysis, yielded a bronze sword ascribed to the Bronze Age(1). It is also known that in the "Sub-

merged Forest Bed" at Rhyl, exposed in 1914, remains of birch, oak, "fir", elm and bracken were present in a peat layer over a pale blue clay containing Neolithic stone implements and animal remains(1).

In the absence, however, of local archaeological evidence any attempt to date the horizons of the Nant Ffrancon deposits must be based primarily on the internal evidence of the pollen diagrams themselves and their comparison with diagrams for other British and European peats. The deep Cwm Idwal peat lends itself as the most complete record for chronological considerations. Only in this is there a well-defined *Pinus* maximum, at about 10 ft. deep: pine trees grew on the spot, as large remains of its timber have been found. Shortly above this horizon there is a rapid and permanent decrease in the importance of pine, leading to its ultimate disappearance in the upper part of the zone already mentioned as represented in the other profiles. The pollen curves for pine and alder, pine decreasing upwards, cross between the 9- and 10-ft. horizons (Fig. 2), a feature that Erdtman describes as indicative of the end of boreal time. If we then look for the general criterion for the end of the subsequent atlantic time, it is found at about 7 ft., where the rising birch curve crosses the alder curve, now decreasing. The change about the 5-ft. level from predominance of tree pollen to predominance of heath pollen may indicate a widespread and marked change of climate, involving a "deterioration" of conditions with increased rainfall and colder average temperatures. The onset of this change would correspond to the transition from subboreal to subatlantic times.

On this scheme the lowest parts of the profile would be boreal, and the period of extensive peat formation in the valley would be placed in the subboreal period. This period, however, is marked in Ireland, parts of Great Britain and Europe by a second *Pinus* maximum. Another difficulty is that the pine maximum here is not accompanied by a hazel maximum, followed by rapid increase in *Quercetum mixtum*, as found elsewhere in Europe in boreal time. The presence of alder, moreover, in the deepest horizons suggests that the peats cannot be earlier than the late boreal. A striking feature of the analyses is the low frequency at all horizons, where found, of oak and its associated pollens. The number of tree species entering into the composition of these post-Glacial woodlands is small. It is possible that all these differences may be explicable as due to altitudinal and geographical factors. If, on the other hand, the base of the Idwal profile is assigned to the second pine period, then the diffi-

culties of the relatively low percentages of hazel and mixed-oak are obviated.

The pine maximum has only been traced in the standard peat; the belt more or less common to the other profiles lies above. If it is assumed that the change from pine woodland to birch-alder woodland represents a major climatological difference, then the optimum conditions of peat formation were apparently attained at the end of the warm subboreal, and in the early subatlantic.

The peculiar feature of a long, narrow and deep lake in the main valley would prevent deposition of peat until it had drained away, probably some considerable time after the retreat of the glaciers. This lake may have lasted until the boreal period. There is no method of determining when it emptied, but it is certain that it would produce marked changes in the micro-climate of the valley and in the drainage of the higher cwms when it disappeared.

It appears, then, that the decision between these two views must await further evidence. All the deposits so far examined lie within the limits of glaciation: there are, however, deposits at higher altitudes above the glaciated rocks, and their examination may yield earlier records. Though the area included in the Nant Ffrancon valley is so restricted, yet the topographical conditions, especially altitude and aspect, are so varied that variations in the rate and duration of deposition cannot be a matter for surprise. It is possible, indeed, that a more intensive and extended comparative study of Snowdonian peats may also throw light on the post-Glacial topographical ecology of this interesting area.

ACKNOWLEDGMENTS

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STUDIES OF THE PHYSIOLOGICAL IMPORTANCE OF THE MINERAL ELEMENTS IN PLANTS

VII. THE EFFECTS OF POTASSIUM AND CHLORIDE IONS ON THE DIASTASE OF BROAD BEAN LEAVES

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(With 7 figures in the text)

INTRODUCTION

It has been shown in previous papers (Cattle, 1933; James and Cattle, 1933) that potassium and chloride ions exercise a marked effect on the diastatic activity of certain of the higher plants. As a result of further work we are now able to describe a little more fully the contrasting ways in which the two ions operate. The work is described below under the following headings: (1) the distribution of potassium ions, chloride ions, and diastatic activity in the foliage of the plant, and the effects of potassium and chloride starvation of the plant on these distributions; (2) the activation by potassium chloride of diastase extracted from normal plants and from plants starved of potassium or chloride ions.

Earlier work had been done using a pure line of broad bean (Sutton's Exhibition Longpod) which grows well in water culture and which normally forms a single stem without side-shoots, and the same variety was used for the work described here. The seeds were graded, sterilised, germinated in sand and finally transferred to culture solutions made up according to formulae in James (1933). In each set of fifty cultures, half the plants were grown in a normal solution and half in solutions deficient in either potassium or chloride. The plants starved of potassium exhibited the well-known starvation symptoms; those starved of chloride were not apparently different from the normal plants. Estimations were confined to the leaves, which were numbered from the apex downwards, leaves from corresponding nodes being treated as one sample. All the cultures were raised in May and June, the most favourable season for growth. When growth was complete the leaves were dried, powdered and stored to await investigation.

I. DISTRIBUTION

(a) *The distribution of potassium ions*

Method. Potassium distribution was determined by the cobalt-nitrite method of Kramer, Tisdall and Kerr, previous experience (James and Penston, 1933) having shown it to be well adapted to the purpose.

Results. Analyses were performed of the leaves from the apex downwards, and the results are expressed in Table I as grams of

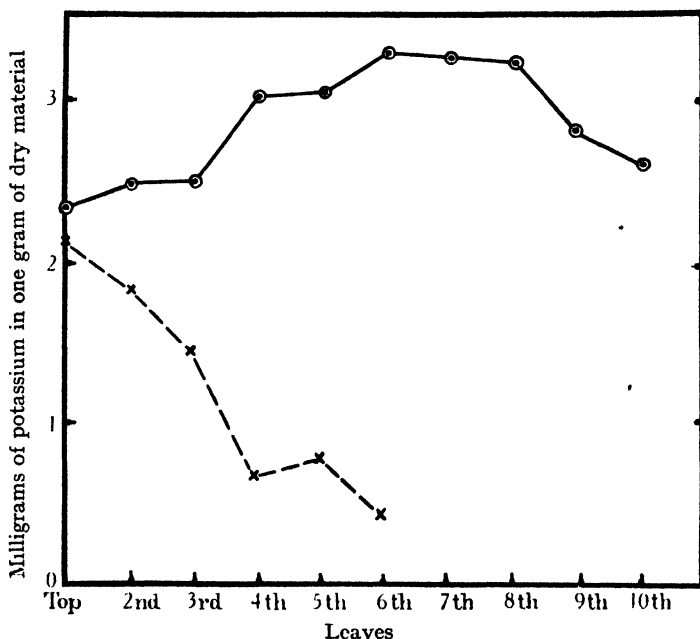


Fig. 1 Distribution of potassium in normal and potassium-starved plants.
 ○—○ Plants grown with complete culture solution.
 × --- × Plants grown without potassium in the culture solution.

potassium in 100 gm. of material dried at 35° C. (see also Fig. 1). This basis was chosen for the sake of uniformity with the diastase results. The cultures were sampled at the usual stage of development, i.e. when the normal plants growing in a complete water-culture solution had ten leaves, and at this time the plants starved of potassium had six leaves, the lower leaves having already fallen away

TABLE I

Leaf	Apex	1	2	3	4	5	6	7	8	9	10	Base
Potassium	Complete	2.31	2.46	2.74	2.99	3.06	3.31	3.28	3.26	2.83	2.63	
% dry	culture											
material	Without	2.12	1.82	1.44	0.69	0.78	0.43	—	—	—	—	
	potassium											

(b) *The distribution of chloride ions*

Method. The standard methods were found to be unsuitable for the estimation of such quantities of chloride as are to be found in plant tissues, and the method eventually adopted is given in detail in a preceding paper (Cattle, 1935). Plant extracts containing chloride are prepared by acid "wet-ashing" in presence of silver nitrate, thereby precipitating the chloride and preventing loss by volatilisation, the principal defect in most methods of chloride estimation in plants. The residual silver nitrate is back-titrated with thiocyanate.

Results. The distribution of chloride was determined for two sets of cultures, viz.:

- (1) Harvested early in June:
 - 25 plants grown in normal solutions.
 - 25 plants grown in solutions deficient in potassium.
- (2) Harvested late in June:
 - 25 plants grown in normal solutions.
 - 25 plants grown in solutions deficient in chloride.

Table II gives the results in summary:

TABLE II

Milligrams of chloride in 1 gm. of dry material

Leaf	...	1	2	3	4	5	6	7	8	9	10
(1) Complete	...	3.4	4.0	5.0	9.4	12.4	14.6	14.8	13.0	13.4	11.4
culture											
Without	...	3.4	5.4	9.6	12.0	10.8	11.2	10.2	—	—	—
potassium											
(2) Complete	...	2.6	4.4	6.0	8.6	9.8	13.2	16.0	17.4	16.2	—
culture											
Without	...	2.4	1.6	2.6	2.0	2.2	1.2	1.0	0.8	1.4	—
chloride											

Two separate chloride extractions were performed on the leaf material from each node, and these values showed very close agreement. Table II is calculated from the averages of the experimental readings. The chloride cultures were harvested at a rather earlier stage in their development than were the potassium cultures—i.e. when only

nine leaves had developed on each plant—but if this slight difference in age of the plants is taken into consideration, the distribution of chloride in the normal (complete) cultures of the two sets shows reasonable agreement (see Figs. 2 and 3).

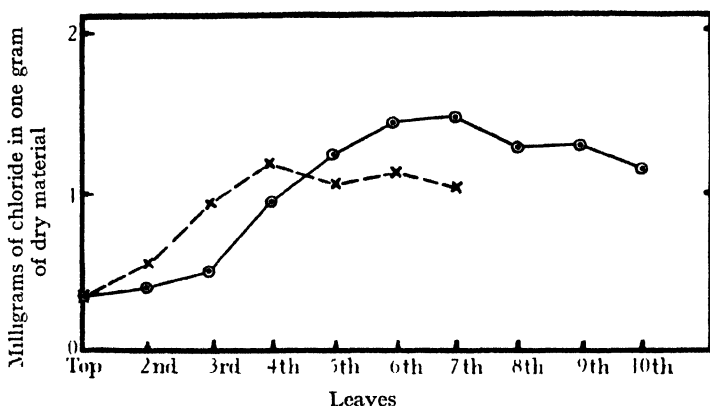


Fig. 2. Distribution of chloride in normal and potassium-starved plants.
 ○ — ○ Plants grown with complete culture solution.
 × --- × Plants grown without potassium in the culture solution.

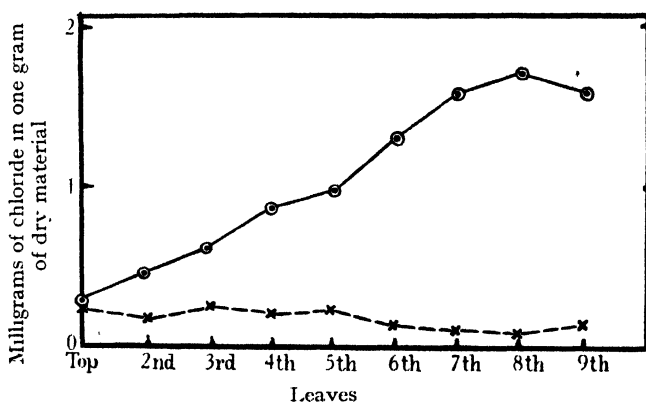


Fig. 3. Distribution of chloride in normal and chloride-starved plants.
 ○ — ○ Plants grown with complete culture solution
 × --- × Plants grown without chloride in the culture solution.

The graph of distribution of chloride in cultures deprived of potassium is of a similar form to that for normal plants, but the highest concentration is shifted several nodes nearer the apex. This is to be expected since there are fewer green leaves and the whole of the life processes are, so to speak, telescoped in the starved plants.

If a comparison is made between the distribution of potassium and that of chloride (see Figs. 1 and 2) in normal plants and those grown without potassium, it is seen that although the distribution of potassium and chloride shows a certain similarity in the normal plants, potassium deficiency affects the potassium and chloride content in very different ways. Potassium content is reduced to progressively lower levels in the leaves, while chloride content shows a definite maximum at the fourth leaf. These results suggest that the distributions of chloride and potassium ions are largely independent, even although a deficiency of potassium in the nutrient solution does lead to a reduction of the chloride content of the leaves as a whole.

A deficiency of chloride in the culture solutions affects the internal distribution of this ion in the way that would be expected (Fig. 3). Chloride content is reduced to a uniformly low value throughout the plant, and the accumulation shown by the older leaves of normal plants is entirely absent from the starved plants.

(c) *The distribution of diastatic activity*

Method. The distribution of diastase was examined by the estimation of sugar formation by the Hagedorn-Jensen micro-titration as modified by Hanes (1929) in a starch digest incubated at 33° C. at pH 6.8. This, it must be pointed out, is not intended as a complete analysis of the whole diastase complex, but only as an attempt to trace the effect of the cultural conditions and the position of the leaf upon the plant on the summation of the processes concerned with the conversion of starch to sugar.

Results. The results are collected in tabular form in Table III, and are in every case the averages of nine readings—three titrations from each of three digests. The experimental values showed close agreement, and the averages may therefore be considered reliable.

TABLE III

*Diastatic activity in terms of thiosulphate equivalent to sugar
formed by 1 gm. dry weight in 1 hour*

Leaf ...	1	2	3	4	5	6	7	8	9	10	11
(1) Complete culture	956.3	1445.7	1418.8	848.6	587.1	812.0	646.5	713.3	378.6	598.1	408.5
Without potassium	1059.6	1010.2	884.5	413.1	377.1	417.0	-	-	—	—	—
(2) Complete culture	1056	—	1254	1364	1003	783	365	200	176	—	—
Without chloride	1096	—	1263	1236	1074	814	603	444	277	-	—

Here, as in the determination of chloride distribution, two sets of cultures were used, one set showing deficiency of potassium, the

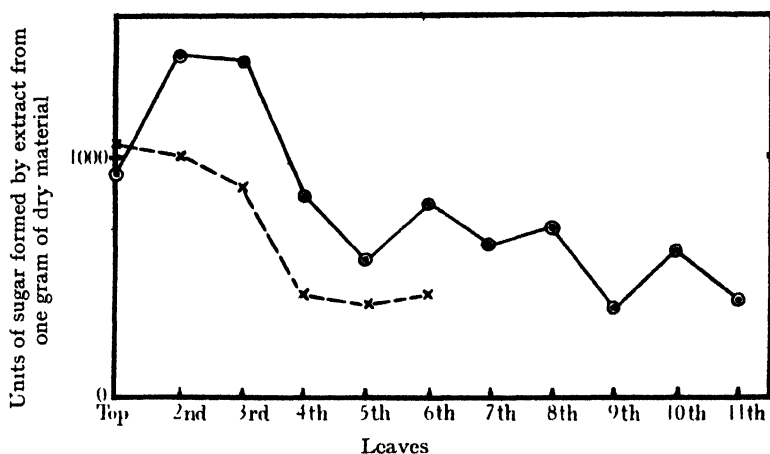


Fig. 4. Distribution of diastase in normal and potassium-starved plants.

○ — ○ Plants grown with complete culture solution.

× --- × Plants grown without potassium in the culture solution.

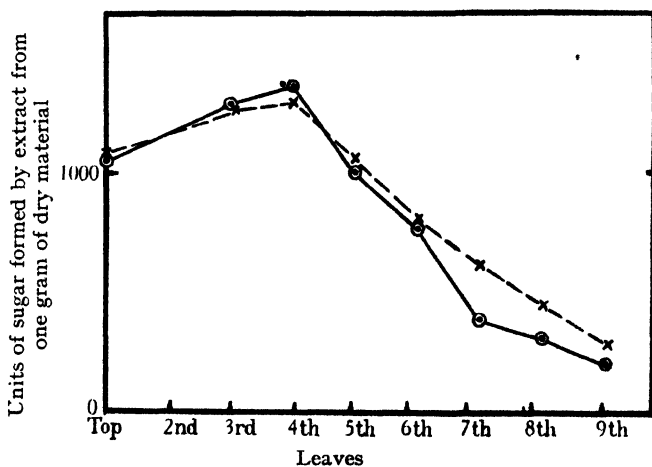


Fig. 5. Distribution of diastase in normal and chloride-starved plants.

○ — ○ Plants grown with complete culture solution.

× --- × Plants grown without chloride in the culture solution.

other of chloride. Samples from the same harvest were used here as for the chloride determinations, and also for the activation results

given in the next section of the paper. The results, therefore, are all directly comparable.

The effect of a deficiency of potassium on diastase distribution is a lowering of the activity in leaves of all ages except at the apex, and there is a suggestion that the position of greatest activity is shifted higher up the plant (Fig. 4). Such a shift is not always seen in potassium-deficient cultures, as for instance in the earlier work on enzyme distribution, and there is a certain amount of evidence that this may be a seasonal effect (Cattle, 1933). Chloride cultural deficiency, on the other hand, has no significant effect on the amount of diastase in the leaves (Fig. 5).

It should be noted that it is the actual amount of enzyme formed by the plant that is measured here, independently of any ionic activating effect. The digest is made up in such proportions as to dilute any ions present in the extract to such an extent that they do not cause activation (see p. 292), although by using the micro-method it is possible to estimate enzymatic activity satisfactorily at such low concentrations.

II. ACTIVATION

In this section of the work, the activation of extracted diastase by the addition of potassium chloride to the digests was determined, and the results are given in the form of activation ratios:

Activity in presence of potassium chloride

Activity in absence of potassium chloride

Method. As this work was actually in progress while the method for estimating chloride was being worked out, no knowledge of the chloride content of the tissues was available and a more or less arbitrary amount of potassium chloride was used. A solution of potassium chloride was added to the digest in such quantities as to bring the concentration of potassium chloride to 0.23 per cent. (0.024*M*) in the mixture. This was known from previous work (James and Cattle, 1933) to be near the optimal concentration for potato diastase, and corresponds roughly with Myrbäck's results for sodium chloride and salivary diastase (0.01–0.1*M*). When the chloride content of the leaves had been determined it was found that the concentration of chloride ions in the digests to which potassium chloride was added was similar to the concentration of chloride ions in the plant sap. An equal volume of water was added to each control digest so that the concentration of diastase was the same in

both. Here, as in the previous section, diastatic activity was investigated only by the rate of sugar production, and other aspects of the question will be dealt with in a later paper.

Activation of the diastase extracted from leaves at each node of the following sets of plants was determined:

- (1) Harvested early in June:
 - 25 plants grown in normal solutions.
 - 25 plants grown in solutions deficient in potassium.
- (2) Harvested late in June:
 - 25 plants grown in normal solutions.
 - 25 plants grown in solutions deficient in chloride.

Results. The activation ratios obtained are given in Table IV. The actual enzyme activity can be seen in Figs. 6 and 7. Addition of potassium chloride activates the diastase in all leaves of all cultures,

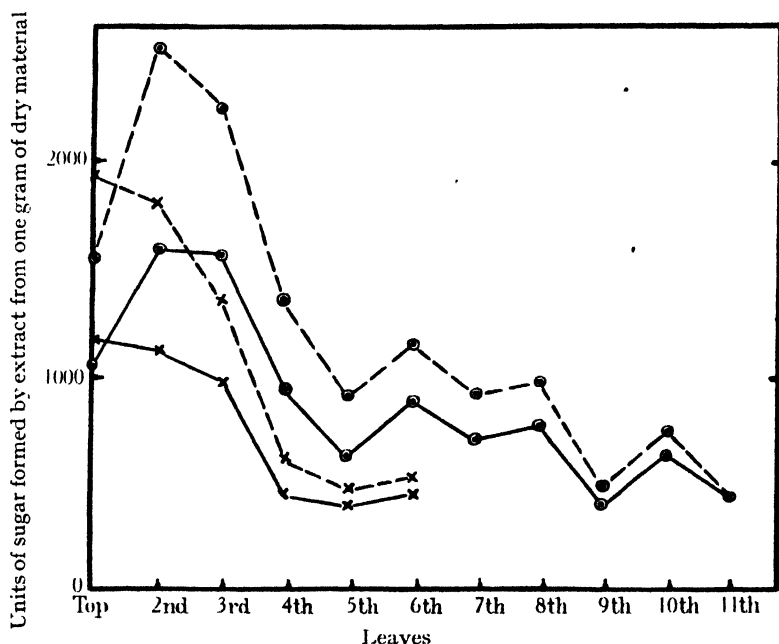


Fig. 6 Effect of addition of potassium chloride to diastase extracted from normal and potassium-starved plants.

●—● Diastase from normal plants; no addition of potassium chloride to digests.

●---● Diastase from normal plants; potassium chloride added.

×—× Diastase from potassium-starved plants; no addition of potassium chloride to digests.

×---× Diastase from potassium-starved plants; potassium chloride added.

though the activation ratio decreases with the age of the leaf and its distance from the apex. It is noticeable that the absence of chloride from the cultures reduces the ratio in practically every leaf, i.e. it limits the power of the diastase to respond to direct activation by the same ion. This is a curious and at present inexplicable result, but has an analogy in Hartt's (1934) experiments with phosphates. Comparisons between leaves receiving and not receiving potassium in

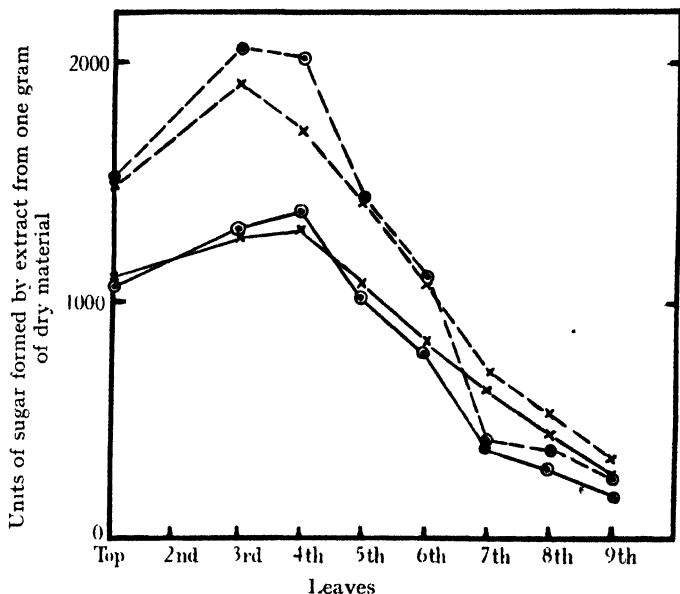


Fig. 7 Effect of addition of potassium chloride to diastase extracted from normal and chloride-starved plants.

- Diastase from normal plants; no addition of potassium chloride to digests
- Diastase from normal plants; potassium chloride added.
- ×---× Diastase from chloride-starved plants, no addition of potassium chloride to digests.
- ×---× Diastase from chloride-starved plants; potassium chloride added.

their culture solutions are difficult owing to the general acceleration of ageing characteristic of potassium starvation. All that can be said is that the activation ratio falls off more rapidly in the starved leaves.

Owing to the instability of diastase when extracted, preparations entirely freed from chloride ions by electro dialysis could not be prepared, but when the results of the chloride analyses were complete it was apparent that the inevitable chloride content of the digests

was very low indeed. With plants starved of chloride it averaged only $2.1 \times 10^{-6} M$, while the corresponding value for plants grown in a complete culture solution was $12.3 \times 10^{-6} M$. Increasing the chloride content of the digests from starved plants to $12.3 \times 10^{-6} M$ did not increase the diastatic activity, the effect of such a concentration being quite unmeasurable.

Comparing the chloride analyses with the corresponding water contents of the tissues concerned allowed the following internal average concentrations to be calculated, since the chloride in plant tissues is all in the water-soluble condition:

	Chloride concentration
Plants grown on complete culture solutions	... 0.045 <i>M</i>
Plants grown on solutions lacking potassium	... 0.034 <i>M</i>
Plants grown on solutions lacking chloride	... 0.009 <i>M</i>

The concentration 0.024 *M* potassium chloride supplied to the activated digests would give therefore an addition comparable with that in the sap of the living tissues and there is a strong probability that chloride activation takes place in the living cells. The *pH*, which affects the degree of activation (Myrbäck, 1926), was approximately the same in the extracts as in the sap, since the natural buffers were employed. The fact that extracts of leaves with low internal chloride concentrations showed no difference of activity from normal leaves is due to the dilution of the extract in preparing the digests.

CONCLUSION

The conclusions that can be drawn from the work described above may be summarised as follows:

(1) Potassium as a nutrient substance, i.e. supplied in the culture medium, increases the diastatic activity of leaves at practically all stages of development, though it is unlikely (Haldane, 1930) that the potassium ion itself when added to the extracted enzyme can bring about activation.

(2) Chloride ions added to the nutrient medium have little or no effect in increasing the diastatic activity of the leaves, but chloride ions added direct to a digest containing their diastase activate the diastase strongly. Increases of 64 per cent. have been recorded with 0.23 per cent. potassium chloride.

(3) Increasing the concentration of chloride ions in the digest to a level comparable with the chloride concentration in the sap and using a similar *pH* produces marked activation of the diastatic

activity. There is, therefore, a good probability that such activation occurs in plants. Reference to the literature confirms the possibility of such an activation. James (1930) showed an increased rate of loss of starch from the leaves of potato plants receiving fertilisers containing chlorides. Giessler (1928) showed an increased rate of disappearance in *Drosera* leaves in contact with chloride solutions. Montfort (1926) obtained similar results with lilac leaves. Other examples might be added, but caution is necessary as similar effects have been ascribed to other ions, both cations and anions, and other interpretations of the data are possible.

(4) Potassium ions, which possess the highest mobility of all cations (except perhaps H^+) inside plants are nevertheless not able to influence seriously the internal distribution of chloride ions (cf. p. 287). They cannot, therefore, cause an indirect activation by behaving as chloride carriers, even when potassium is present in abundance. We must, therefore, suppose that the effect of nutrient potassium is to increase the amount of diastase itself that is formed.

(5) Colloidal precipitates from extracts of plant materials usually contain diastase, but we have ourselves observed that they may be entirely free from potassium. Kostytschew and Eliasberg (1920) have also shown that lead acetate and tannin precipitates of leaf extracts contain no potassium. It, therefore, seems improbable that potassium should enter into the structure of the diastase molecule. We can only conclude that directly or indirectly potassium is a catalyst at some stage in the synthesis of a part of the diastase complex. In the complete absence of any knowledge of these formation stages the potassium effect cannot at present be more clearly defined.

SUMMARY

The effects of potassium and chloride ions on the behaviour of the diastase of broad-bean leaves is examined in a number of ways, and it is concluded that while the chloride ion may act as a direct activator of preformed diastase, potassium is more likely to act as a catalyst at some stage in the building up of the diastase complex.

The work reported in this paper was carried out with the help of a grant from the Department of Scientific and Industrial Research, to which we desire to express our grateful thanks.

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STUDIES OF THE PHYSIOLOGICAL IMPORTANCE OF THE MINERAL ELEMENTS IN PLANTS

VIII. THE VARIATION IN POTASSIUM CONTENT OF POTATO LEAVES DURING THE DAY

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(With 5 figures in the text)

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I. INTRODUCTION

WHILST carrying out experimental work on the seasonal variation in potassium content of the haulm and leaves of the potato plant, evidence was obtained of a possible fluctuation in the potassium content of leaves during the day.

In view of the probable importance of potassium in leaf functions, it seemed worth while to verify this point, and to attempt some correlation between change in potassium content, if such were found, and other factors such as water content and dry weight.

II. METHODS

The variety of potato, Majestic, which had been employed in the previous work, was again used in these experiments.

Samples for each experiment were collected as follows: leaves of the same age were gathered in lots of 35-40 leaves, at different times of the day. As soon as a sample was collected and weighed, each leaf was separated into blade and stalk portions—the stalk consisting of

the petiole and the midrib extending between the leaflets—and the fresh weight of the two portions then determined separately; no movement of potassium or other materials out of the blade into the stalk, or *vice versa*, between sampling and drying in the laboratory could therefore take place.

At the end of the day the samples were removed to the laboratory and dried in an electric oven at 100° C. Ash and potassium were subsequently determined, the potassium being estimated by the cobalti-nitrite method already described (James and Penston (1933)).

The experiments were carried out in August, late in the season when the plants were mature. Of the three recorded here, one was made in 1932, the others in 1933. The age of the leaf was not the same in each experiment: in 1932 on August 12th the seventh leaf down from the apex of the highest lateral was taken; in 1933 the eighth and the twelfth leaf respectively were used in the samples collected on August 21st and 25th. The difference in the age of the leaves is indicated by a comparison of their average areas: 7th leaf, 32 sq. cm.; 8th leaf, 25 sq. cm.; 12th leaf, 41 sq. cm.

The stage of development of the plants as a whole was, however, about the same, although one lot of plants was collected in 1932 and the others a year later. The plants had reached maturity, the tubers were well developed and nearly ready for lifting. Some of the lower leaves were yellow, but all those used in the samples were still green.

As the changes in the dry weight and water content of the samples throughout the day are to a large extent determined by the photosynthetic and transpiratory activity, two functions which are affected by the external conditions, a few facts regarding the weather conditions will not be out of place.

On August 12th, 1932, and August 25th, 1933, conditions were similar, both days being hot and sunny, except for a short, rather severe shower on the 12th, at about noon (G.M.T.), and later a gradual clouding over towards the end of the day, with rain after 6.30 p.m. August 21st, 1933, was, on the other hand, dull and overcast most of the time, and the atmosphere rather heavy, although temperature remained relatively high; rain started to fall at about 1 p.m. and soon put an end to the experiment. The maximum temperature on each day was reached at about 1 p.m., this followed a lower morning maximum at about 10 a.m., the temperature between 11 a.m. and 12 noon being less than at 10 a.m.

III. EXPERIMENTAL RESULTS

(i) *Potassium, ash, dry weight and water content of leaves*

The results for the three experiments are given in Table I. The figures represent absolute weights in grams per 100 leaves for the blade portions.

An examination of the table will show considerable variation in

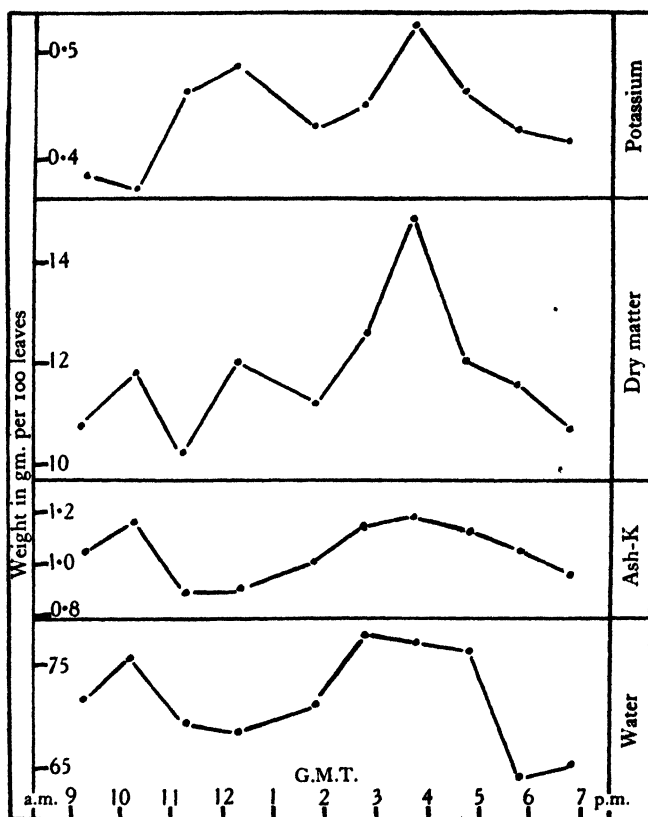


Fig. 1. Graphs showing variations in water, dry matter, potassium and residual ash content of samples of potato leaves, on August 12th, 1932. Results expressed in grams per 100 leaves.

all the four quantities determined. The nature of these variations for Exps. I and II, those made on sunny days, are more clearly shown in graphical form in Figs. 1 and 2.

Although varying in detail, the curves all show very clearly

one striking feature of similarity, namely, that the maxima for water, dry matter, residual ash and potassium, coincide at some time between the period 3-4 p.m., in both experiments. And this

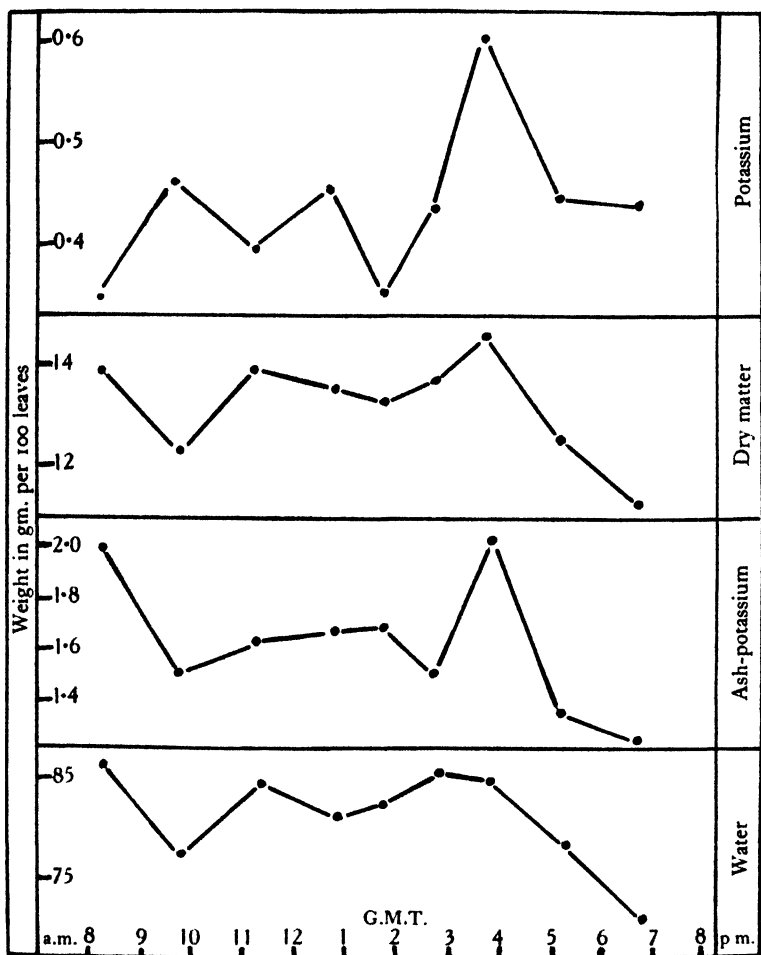


Fig. 2 Graphs showing variations in water, dry weight, potassium and ash content of potato leaves, on August 25th, 1933. Results in grams per 100 leaves.

maximum is followed by a steady decline towards the evening. Potassium and dry weight curves show some similarity over the morning period, each having a secondary morning maximum, followed by a midday fall, before the greater increase in weight in the afternoon.

There is also an interesting similarity in the appearance of the graphs representing potassium content and dry weight, and one published by Davis and Sawyer (1916) showing variation in starch content of potato leaves.

TABLE I

*Water, dry matter, potassium and residual ash, in potato leaves
(weight in grams per 100 leaves)*

Exp. I, August 12th, 1932				
Time	H ₂ O	Dry weight	Ash-K	K
9.20 a.m.	72.80	10.80	1.033	0.382
10.20	76.50	11.90	1.143	0.367
11.20	69.88	10.20	0.877	0.463
12.0 noon	69.5	12.10	0.894	0.486
1.45 p.m.	71.73	11.27	1.003	0.427
2.45	78.40	12.60	1.132	0.448
3.45	77.70	14.80	1.167	0.528
4.45	76.80	12.00	1.118	0.457
5.45	64.68	11.62	1.038	0.422
6.45	66.00	10.70	0.968	0.412
Exp. II, August 21st, 1933				
Time	H ₂ O	Dry weight	Ash-K	. K
8.45 a.m.	58.84	10.09	1.295	0.315
10.25	76.99	11.48	1.812	0.448
11.55	57.79	9.73	1.305	0.355
12.55 p.m.	77.57	12.11	1.823	0.397
1.55	82.23	13.38	2.143	0.357
3.0	70.84	12.16	1.554	0.346
4.35	91.55	11.67	1.451	0.359
Exp. III, August 25th, 1933				
Time	H ₂ O	Dry weight	Ash-K	K
8.15 a.m.	86.02	13.96	1.973	0.357
9.45	77.24	12.28	1.490	0.459
11.15	84.51	13.90	1.615	0.395
12.45 p.m.	81.56	13.55	1.647	0.453
1.45	82.22	13.27	1.671	0.349
2.45	85.57	13.68	1.488	0.482
3.45	84.91	14.55	2.055	0.605
5.15	78.42	12.54	1.337	0.443
6.45	72.30	11.35	1.219	0.431

Of all the quantities examined, the variation in potassium is the most considerable, the difference between its maximum and minimum content in Exp. II being as much as 73.0 per cent., the total residual ash in the same experiment shows a maximum variation of 68 per cent.

Some at least of the residual ash elements must be in combination in the cell structure, e.g. Ca, Mg, P, S. If, therefore, the changes in residual ash content are due to fluctuations in free water-soluble constituents only, their maximum variations may be as considerable

as that of potassium, which is usually considered to be all loosely combined. One interesting point is that the total ash in Exp. II, 1933 is about 50 per cent. greater than in Exp. I, 1932, though the potassium content of the two leaves is of the same order. The 1933 leaf is the older, therefore more ash is probably either built up into its permanent structure, or deposited in some way as waste in the cells, and so may not be free to re-enter the mineral cycle.

In view of the function of the leaf, it is to be expected that dry weight and water content should vary, but that potassium and residual ash should also show such tremendous fluctuations is rather remarkable and very suggestive.

(ii) *Comparison of water entering leaf and potassium*

During the same day that samples of leaves were being collected, data were obtained to show the rate at which water was being lost from the same leaf surface by transpiration. The cobalt chloride method was used. Pure filter paper, impregnated with 1 per cent. cobalt chloride, was cut into pieces about 0.5 mm. sq.; by preliminary tests, those pieces only which changed from one standard tint to the other in the same time were selected for use. Further tests, by obtaining the increase in weight of lots of 50 pieces, which had been held in saturated atmosphere until change in colour had been effected, gave the average amount of water absorbed by a single piece during the change between the two colour standards. Hence it was possible to determine the loss of water from both surfaces of the leaf quantitatively. Transpiration readings were taken immediately before each sample was collected. Several cobalt determinations were made on the sample leaf from different plants on each occasion, and the average area of the leaf being known a rough estimate of the amount of water being lost per 100 leaves per minute at that particular time could be obtained. These results are recorded in Table II.

During the day of August 12th, as has been mentioned, there was considerable sunshine and a high temperature, except for a short shower between 12 noon and 1.15 p.m., and general clouding over towards the evening when rain again fell after the last sample had been collected at about 7 p.m. The sun was sufficiently powerful to dry the surface rain water off the leaves after the midday shower before the 1.30 p.m. transpiration reading was made, and although there must have been fluctuations in the transpiration rate between 12 noon and 1.30 p.m., this is not very obvious from the slight difference between the readings before and after the shower. The

approaching evening rain, coinciding with evening loss of light and temperature, does, however, result in a considerable fall in the transpiration rate at 6.30 p.m.

TABLE II

The transpiration rate, expressed as grams of water lost per minute per 100 leaves, measured just before the collection of samples for analysis in the three experiments.

Exp. I, August 12th, 1932		Exp. II, August 21st, 1933		Exp. III, August 25th, 1933	
Time	Tran- spiration rate gm.	Time	Tran- spiration rate gm.	Time	Tran- spiration rate gm.
9.0 a.m.	4.92	8.30 a.m.	0.426	8.0 a.m.	4.97
10.0	7.49	10.0	0.453	9.30	3.48
11.0	6.47	11.45	0.390	11.0	3.59
12.0 noon	6.47	12.45 p.m.	0.483	12.30 p.m.	2.87
1.0 p.m.	5.45	1.45	0.486	1.30	4.31
2.30	5.45	2.45	0.468	2.30	5.10
3.30	5.17			3.30	5.88
4.30	3.76			5.0	3.56
5.30	3.04			6.30	2.93
6.30	1.48				

On August 21st, 1933, rain fell soon after 3 p.m., thus putting an end to the experiment. No transpiration reading was made after 3 p.m., but one more sample was collected for analysis, and it will be seen from Table I that the leaf water content had risen considerably in this last sample, as was to be expected. In Exps. I and III, both on sunny days, the transpiration loss is considerable; but in Exp. II, on the dull day, the rate is very much less, being in fact of the order of one-tenth of that on a sunny day, when the results are expressed, as in the table, as grams of water lost per minute per 100 leaves. If, on the other hand, the transpiration rate is expressed as loss of water per unit area of 1 sq. m. of lower leaf surface, the figures for the first reading on each day would be: Exp. I, 14.0 gm.; Exp. II, 1.13 gm.; and Exp. III, 8.03 gm.; because the average areas of the three leaves is different. As the other quantities measured and given in Table I are all expressed on the basis of weight per 100 leaves, it seemed best to record the transpiration results also in the same manner.

An exact comparison is out of the question, but in order to obtain some idea of the relationship between movement of potassium in the leaf during the day and the movement of water, calculations were made of the change in rate of potassium accumulation and total water entering the leaf (including that lost by transpiration),

assuming that the rate of change was uniform, and using the figures given in Tables I and II.

For example: in Exp. III, between 8.15 and 9.45 a.m. the leaf water content fell by 8.783 gm., and the potassium content increased by 0.1025 gm. per 100 leaves. The period of time represented is 90 min., therefore the rates per hour are: two-thirds of 8.783 and two-thirds of 0.1025 gm., i.e. 5.855 and 0.683 respectively. At 8.0 a.m. the transpiration rate was 4.97 gm. per min., at 9.30 a.m. 3.48 gm., and at 11.0 a.m. 3.48 gm.; therefore from 8.15 to 9.45 a.m. the amount of water lost was:

$$\frac{1}{2} [75 (4.97 + 3.48) + 15 (3.48 + 3.59)],$$

i.e. 369.64 gm., or a rate of 246.4 gm. per hour. The total change in the entry of water into the leaf is therefore 246.4 gm. lost by transpiration minus 5.86 gm. decrease in leaf water content, that is, a total change of 240.5 gm. per hour. Figures, for Exp. III, based on such calculations, are given in Table III.

TABLE III

Rate of change in water and potassium in the leaf, during the periods between samples, expressed as grams lost per hour

Time period between samples	Potassium		Water		
	Change in content of leaf cells	Rate of change in gm. per hour	Change in content of leaf cells	Transpiration loss	Rate of change in gm. per hour
8.15-9.45	+0.1025	+0.0683	-8.783	+369.64	+240.6
9.45-11.15	-0.0645	-0.0430	+7.270	+313.31	+212.7
11.15-12.45	+0.0580	+0.0387	-2.950	+295.65	+195.1
12.45-1.45	-0.1039	-0.1039	+0.660	+231.86	+232.5
1.45-2.45	+0.1329	+0.1329	+3.350	+293.90	+297.3
2.45-3.45	+0.1230	+0.1230	-0.660	+317.81	+317.2
3.45-5.15	-0.1020	-0.1080	-6.490	+402.48	+264.0
5.15-8.45	-0.0110	-0.007	-6.12	+287.35	+187.5

The changes in rate are represented graphically in Fig. 3. It is clear that the changes in the two quantities are not proportional throughout the day: but considering the nature of the two processes involved—absorption of water by the roots and loss by the leaves, in the one case, and absorption and movement of ions in the other—exact similarity is not to be expected. The differences may be due to fluctuations in export rate of the potassium or in the rate at which potassium is being fed into the transpiration stream at the root end, or in the stem; the factors governing which cannot at the present be estimated adequately.

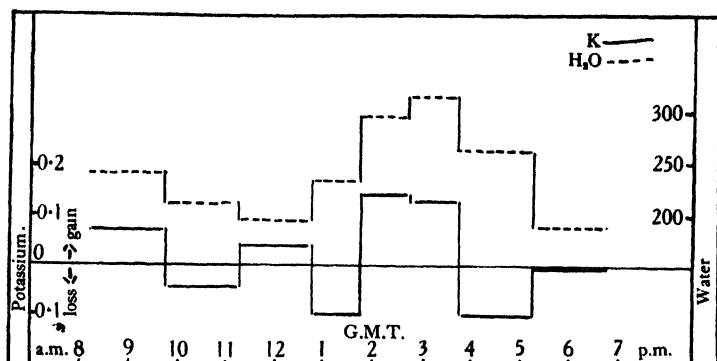


Fig. 3. Comparison of the rate of movement of potassium and water (including that lost by transpiration) in potato leaves, on August 25th, 1933. Rate in grams per 100 leaves per hour.

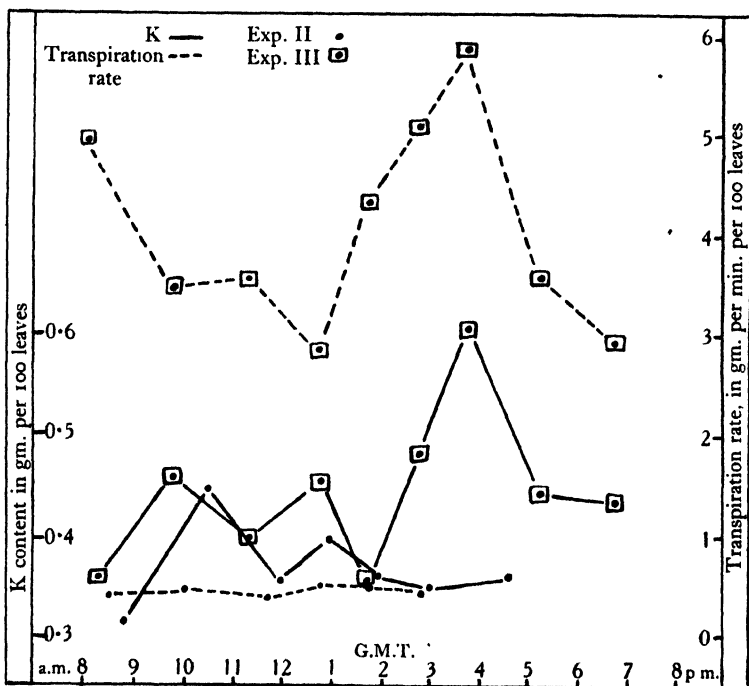


Fig. 4. Potassium content of potato leaves on a dull day during which transpiration is low (Exp. II, August 21st, 1933) compared with potassium content on a sunny day with high transpiration loss (Exp. III, August 25th, 1933).

One interesting feature is that the value of the maximum variation of potassium during the day in the leaf does seem to be related to the rate of transpiration. On a sunny day, with high transpiration, the maximum potassium content between 3 and 4 p.m. is considerable; on a dull day with slow transpiration, fluctuation of potassium on the whole is less obvious, and a maximum between 3 and 4 p.m. scarcely apparent (see Fig. 4).

(iii) *Potassium content of lateral shoots*

Another significant result is that the movement of potassium is over considerable distances, at least as regards any one axillary shoot: samples were taken of a lateral shoot in the morning at about 10 a.m. and transpiration readings were made at the same time on alternate leaves of several similar laterals. At 1.30 p.m. another sample was collected, and further transpiration readings made. The results are given in Table IV. Only the alternate leaves and the stem internode immediately below them were analysed. The total potassium content of the portions analysed was found to have increased during the period between the collection of the two samples. There can be little doubt, therefore, that the potassium content of the lateral stem as a whole had increased. This means that the changes in potassium content cannot be due merely to fluctuations between leaf and adjacent stem tissues, but that they represent some movement at least, from lower regions of the stem or root in the case of the potato plant.

TABLE IV

Potassium content, in grams per 100, of a selection of leaves and internodes from the two sets of lateral shoots collected on August 28th, 1933, at 10 a.m. and 1.30 p.m., together with transpiration rates of the same leaves.

Time	Potassium content of	No. of leaf and internode, counting down from the apex							Total
		Bud	4	6	8	10	14	16	
10 30 a.m.	(i) Leaf	—	0.116	0.410	0.515	0.335	0.418	0.286	2.080
	(ii) Stem	0.161	0.230	0.230	0.285	0.532	0.843	0.624	2.783
	Total	0.161	0.224	0.640	0.80	0.867	1.216	0.901	4.863
1 30 p.m.	(i) Leaf	—	0.083	0.287	0.644	0.595	0.714	0.355	2.678
	(ii) Stem	0.106	0.085	0.274	0.442	0.538	0.465	0.727	2.637
	Total	0.106	0.168	0.561	1.086	1.133	1.179	1.082	5.315
10 30 a.m.	Rate of	—	1.267	1.785	2.005	3.60	2.51	1.14	—
1.30 p.m.	transpiration	—	1.464	1.550	1.960	2.20	1.73	0.445	—

It was not known at the time these samples were collected that a midday minimum occurred for the quantities analysed; hence if samples had been collected between 3 and 4 p.m., it is most probable that the difference in potassium content shown in the results of the two samples taken would have been exceeded. As it is, the difference represents an accumulation in the shoot of about 10 per cent. of potassium. It is interesting to note that the increase is localised mainly in that region of the stem where transpiration is greatest.

(iv) *Potassium in relation to dry weight and water content*

Before proceeding to the discussion of the results, there is one further point that deserves attention, namely the relation of potassium to dry weight and water content, expressed in terms of per-

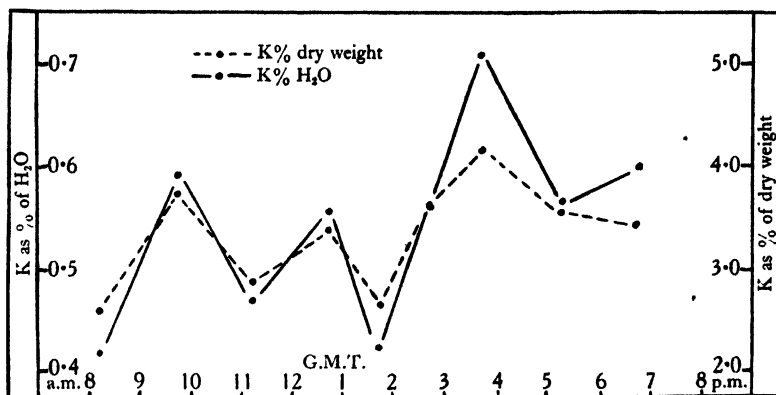


Fig. 5 Potassium content of potato leaves collected on August 25th, expressed as a percentage of (i) dry weight, and (ii) water content.

centages, which have been calculated from the figures in Table I. A graph of the variations in these percentages is seen in Fig. 5 for Exp. III.

It will be observed that the percentages do not remain constant. But a glance at Figs. 1 and 2 will show that while dry weight and water content are varying throughout the day as well as potassium, the latter is accumulating to a greater extent than the two former quantities; hence the percentage value rises markedly between 3 and 4 p.m. As most of the potassium is present in solution, it means that the increase in concentration of potassium in the cell-sap is considerable during the period of greatest photosynthetic activity. This point will be referred to later in the discussion.

IV. DISCUSSION

The purpose of these experiments was to determine the variation, if any, in the potassium content of potato leaves during the day. The result is quite definite: potassium and other ash elements accumulate in the leaf during the hours of sunlight and high transpiration. For example, in Exp. III the difference between the maximum and minimum content during the day is some 73.2 per cent. in the case of potassium and 68.6 per cent. in the case of the residual ash, while dry weight and water content increase by 28.0 and 18.5 per cent. respectively.

This is the main fact emerging from the results. Two points of interest arise from it: what is (1) the main path of translocation of potassium in the plant, and (2) the mechanism of the entry of ions into the cell?

Dealing with these points in order, evidence of the movement of ions is in keeping with the researches of Mason and Maskell (1931) on translocation of mineral elements in the cotton plant, and agrees also with the microchemical and other evidence reported in previous papers of this series (iii, iv). The conclusions that mineral elements are brought into the leaf in the transpiration stream and re-exported in some such channel as the phloem, would seem to be a reasonable one in the present case, because there is evidence of a daily circulation of potassium. Not only is this element continuously entering the leaf and accumulating there during the time that loss of water is known to be proceeding rapidly, but it is also being exported from the leaf, the loss becoming apparent with the fall in potassium content from 4 p.m. onwards when transpiration is slowing down towards the evening.

The second point is not so easy to discuss. In quantitative determinations such as these on the presence of potassium in green leaves, one can indicate correlations with the physiological functions carried on in the organ, but one cannot obtain direct evidence of the forces controlling the accumulation of ions in the cells and tissues.

Recent work, particularly that of Steward and his co-workers (1932), shows that emphasis should be laid on the part played by metabolic activity in interpreting the accumulation of salts in the case of storage tissues. It is possible that a solution along similar lines may eventually be obtained to explain the marked increase in potassium in potato leaves during the day-time.

In section III (iv) of this paper, the relationship between potas-

sium expressed as percentage of dry weight and potassium as percentage of water content has been shown graphically, the two percentage values showing a definite increase at the time of day when dry weight is increasing due to carbon assimilation. This may mean that the potassium accumulation is being controlled primarily by metabolic activity, and the resultant increase in potassium concentration of the cell sap is in its turn affecting the water relations of the cell by raising the osmotic pressure.

During the last summer observations have been made on the osmotic pressure of the potato leaf during the day together with ash and potassium determinations. It is hoped, therefore, to discuss the problem raised by the present experiments at greater length when recording these osmotic results.

V. SUMMARY AND CONCLUSIONS

1. There is a pronounced variation in the potassium content of potato leaves during the day.

2. The general tendency is slowly to increase to a maximum about 3-4 p.m. (G.M.T.), followed by a fall at night.

3. Maximum potassium coincides with maximum dry weight, water content and total residual ash weight, the minima coming about midday.

4. Fluctuations in potassium content are damped down when transpiration is slow.

5. It may be concluded that the potassium is being continuously brought into the leaf, presumably in the transpiration stream, and re-exported in the phloem, because a loss of potassium becomes apparent when transpiration slows down in the evening.

6. The rate of entry of water and the rates of accumulation and of loss of potassium do not seem to be proportional through the day. This may be due to fluctuations in the export rate, or in the rate at which potassium is being fed into the transpiration stream.

7. A tentative reference is made to the probable method of accumulation of ions in the leaf cells, and the effect on osmotic relations.

The writer's thanks are due to Prof. Gates, in whose department this work was carried out, and who provided the ground for growing the plants in his experimental garden at Regent's Park; and to Dr W. O. James, for his help and suggestions in preparing the paper.

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THE DEVELOPMENT AND MORPHOLOGY OF THE LIGULE IN GRASSES

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(With 6 figures in the text)

THE homologies between the various regions of the monocotyledonous and dicotyledonous leaf have been the subjects of much debate among morphologists. It has been questioned whether true stipules are confined to dicotyledons, or whether when similar structures are found in monocotyledonous leaves they can be considered as homologous structures. Lubbock (1894) in a general survey of stipules doubts whether there are any true representatives in the monocotyledons, but nevertheless describes a number of leaves from that group with paired lateral appendages. Although he describes the ochrea of *Potamogeton* he does not include an account of any ligular structure. Other authors, however, had no hesitation in denominating these paired structures as stipules, and some would go further and assert that the ligule represents such stipules fused together. Thus Tyler (1897) considered the lateral part of the leaf rudiment to represent potentially the stipules, the ligule, the ochrea, the lamina of the sheathed petiole, or any other lateral basal segment of the leaf.

One of the difficulties in considering the ligule as formed from fused stipules is its position at the top of the sheath instead of at its base. This difficulty was met by suggesting that the sheath was formed by the fusion of the stipules to the petiole and that the ligule represented the distal ends of the stipules, free from the petiole but fused together. Glück (1901) supported this view in his survey of the stipules of the monocotyledons. He describes the stipules in this group under three heads; stipulae laterales, stipula adnata, and stipula axillaris. The first, with paired basal appendages, conforms more or less to the type of stipule found in the dicotyledons. The second group, in which the stipules are considered to be fused to a greater or less extent with the petiole, includes the grass ligule. The last group is a small one with representatives in the genera *Potamogeton* and *Zanichellia*.

Domin (1911) gives an account of the morphology of the stipule throughout archigonioid plants, and follows Glück in his interpretation of the morphology of the ligule.

Colomb (1887) had much earlier reviewed the morphology of the angiosperm stipule. Not content with external morphology, he investigated the vascular supply of the stipules in many cases. As a result of his investigations with dicotyledons he concluded that the stipular nerves are always branches from the foliar bundles. This condition may be obscured by the foliar bundle being absent above its branch so that all connection with the bundles of the limb is apparently lost. Colomb applies this interpretation of the stipular bundles directly to the grass ligule, and found a disposition of bundles

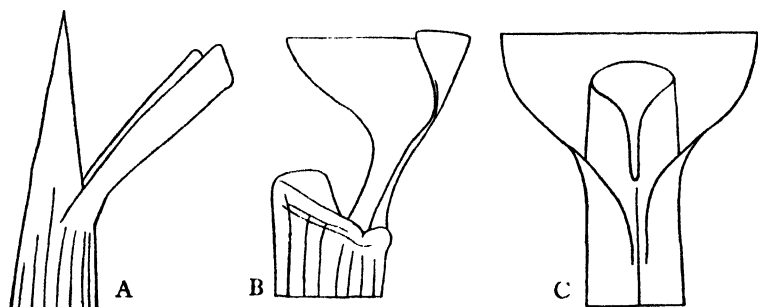


Fig. 1. Ligules. A, *Deschampsia caespitosa*, $\times 3$. B, *Phyllostachys aurea*, $\times 5$. C, *Melica altissima*, $\times 3$.

which led him to conclude that the stipule is represented there. His conclusions were based on the nervation of *Arundinaria japonica* and *Oryza sativa*. In Fig. 1 B and Fig. 6, 3 is represented the ligule of the bamboo *Phyllostachys aurea* and its vascular supply, which is essentially the same as that in the species investigated by Colomb. Interpreting this vascular supply in the same way as that of a dicotyledonous stipule, Colomb considered the transverse bundle (*b*) to be a stipular branch of the foliar bundle (*g*). Colomb distinguished three regions in the typical grass ligule. Firstly, upward prolongations of the margins of the sheath; secondly, a median upgrowth of the adaxial surface of the leaf joining these; and thirdly, at the junction of the median and sheath regions a ridge of tissue in which the stipular nerve runs. This ridge or "talon" he interpreted as a true stipule. After the development of these parts has been described, a different interpretation will be advanced.

Bugnon (1921) gives a detailed account of previous work on the

ligule and describes the development of the ligule in *Dactylis glomerata*. In this species the ligule is formed entirely by an upgrowth of the ventral epidermis of the leaf at the junction of sheath and blade, and is entirely non-vascular; he concludes that it is purely an accessory structure. Ponzo (1931) also concludes that the ligule is an appendage originating as an upgrowth of the ventral epidermis. That the ligule develops only from the surface layer of cells is doubtful evidence of its accessory nature, because the dermatogen seems to be of greater importance in grasses than in other families, probably giving rise to the whole leaf (Bugnon, 1924; Arber, 1934). Bugnon considers that the grass sheath was primitively closed, but that, when the insertion of the leaf is spiral, the overlapping margins of the sheath can take a more important part in the formation of the ligule, and develop vascular bundles of their own. But even in grasses with a completely closed sheath the upper border of the sheath may be included in the ligule and may be nerved as in *Melica altissima* (Fig. 1 C). Bugnon (1921) suggests when discussing the morphology of the lemma, that the ligular nerves are laid down late in the development of the leaf, and states that (p. 62) "le développement de la nervation ligulaire reste un phénomène tardif au cours du développement de la feuille, sans retentissement appréciable sur l'ensemble de l'organisation libéroligneuse foliaire, déjà établie dans son grands traits". As no detailed work has been published on the development of a ligule with a vascular supply, I have investigated the formation of the ligule and its vascular bundles in the case of *Deschampsia caespitosa*. The development of the ligule of *Melica altissima* was also studied to show the inclusion of the upper border of the sheath in a closed ligule. Finally the bamboo, *Phyllostachys aurea*, was investigated in the hope of obtaining evidence for or against Colomb's interpretation of the ligule. After describing the results of these investigations, the conclusions of the authors reviewed above will be re-examined and an interpretation of the morphology of the ligule advanced which seems most closely to fit all the facts of development and adult morphology.

DESCHAMPSIA CAESPITOSA BEAUV.

A number of actively growing stem apices of this species were fixed in Land's mixture of formalin, acetic acid and alcohol and, after embedding in paraffin wax, were cut into serial sections, 10 μ in thickness. The sections were stained in Flemming's triple stain modified for anatomical instead of cytological work by the sub-

stitution of Bismarck brown for safranin. This modification was probably first used by Ethel Sergeant, and I am very grateful to Mrs Arber for acquainting me with this technique. From an examination of this material, supplemented by dissections of stem apices under the binocular microscope, it is possible to trace the development of the vascular tissue of the leaf.

The median bundle is the first to become evident as a cambial strand, its origin being in the axis below the insertion of the leaf rudiment (Fig. 2, 1 *M*). This strand is soon flanked on each side, mid-way between itself and the margin of the leaf rudiment by a lateral cambial strand (Fig. 2, 1 *L*). These strands also originate within the axis, and none of the three strands has at this stage extended into the leaf rudiment which is now a crescentic ridge encircling about half the stem apex (Fig. 2, 3). The next pair of strands to be laid down in each half of the leaf appear practically simultaneously one on each side of the lateral strand, but in all cases when it is possible to detect any difference in the time of their origin it is the marginal strand which forms earlier. In the series of sections from which the figures were taken these pairs of strands are only shown in a well-developed condition (Fig. 2, 1 *m*, *i*), but in other series it is possible to make out that their origin is below the leaf insertion, and that they become differentiated very soon after the lateral strands.

Another pair of strands forms on each side, originating in the leaf rudiment itself, as do all subsequent strands. Again one of the strands is marginal (Fig. 2, 1 *m'*) and the other next the mid-rib (Fig. 2, 1 *i'*), and again it is the marginal which tends, much more markedly in this case, to be laid down first. When this pair of strands first appears, making five vascular strands on each side of the mid-rib, the sheath is very short and cylindrical with the margins fused, at least below, and the transition from sheath to blade is marked only by the furrowing of the ventral surface of the latter, there being as yet no visible trace of the ligule. Before the next strands differentiate the sheath begins to elongate rapidly and its margins continue to develop, overlapping considerably. At the same time the ligule appears as an upgrowth of the ventral surface at the junction of sheath and blade (Fig. 2, 5 *lig*).

The subsequent development of the vascular strands varies slightly in different leaves. Another pair of strands appears in each half of the leaf, a marginal and an intermediate (Fig. 2, 1 *m''*, *i''*). The position of the intermediate varies, being on either one side or

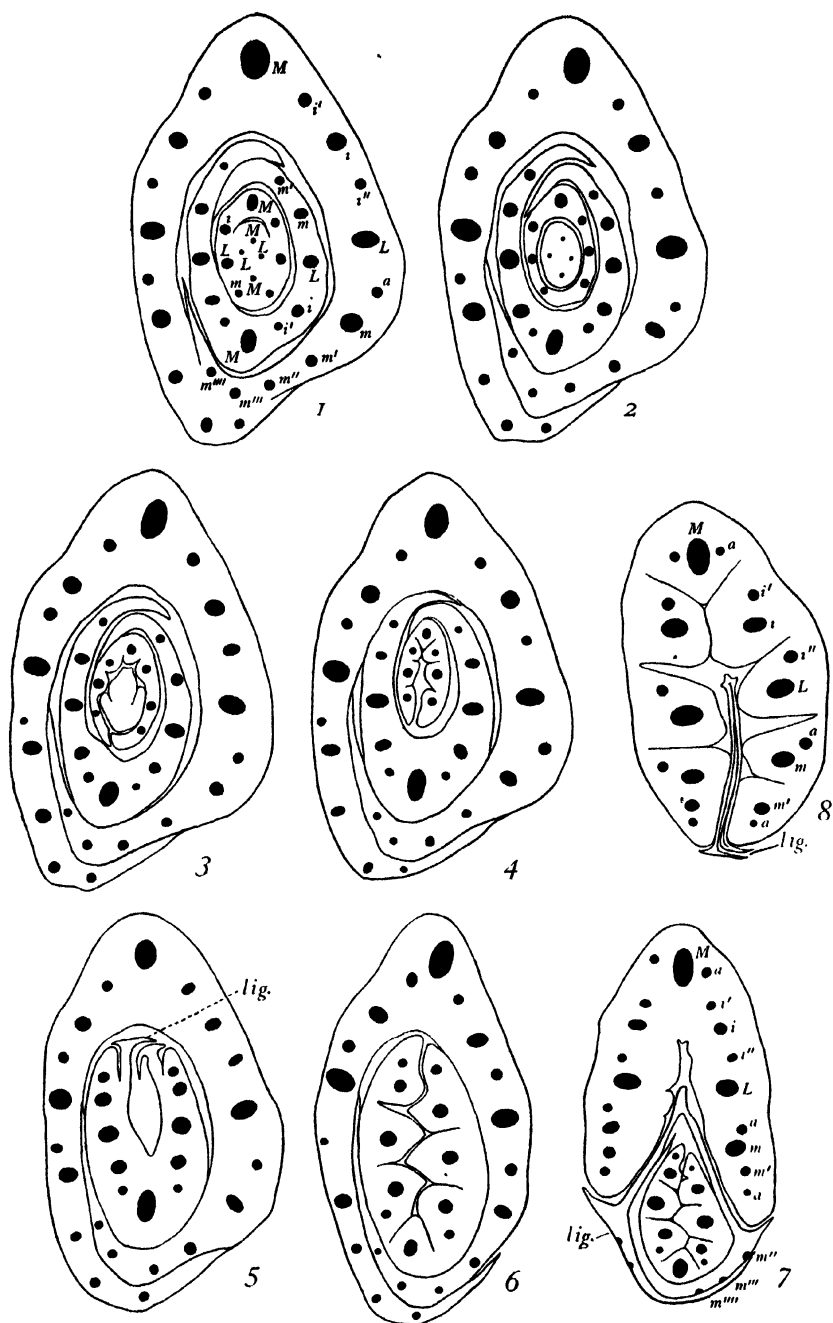


Fig. 2. *Deschampsia caespitosa*. Transverse sections through stem apex and young leaves, $\times 50$. lig., ligule; M, mid-rib; L, first lateral nerve; i, i', i'', successive intermediate nerves; m, m', m'', etc., successive marginal nerves; a, additional nerves of the blade, one entering sheath.

other of the first intermediate to be laid down, but is usually in the position shown in the series figured. The marginal strand originates even more markedly before the intermediate than in the previous pair of strands, and, since the later the origin of a cambial strand the higher it is formed within the leaf rudiment, the intermediate strand of this pair originates considerably higher in the leaf than the marginal, usually above the sheath region in the base of the blade. With the continued elongation of the sheath the marginal and intermediate strands of this pair may become widely separated, the marginal remaining in the sheath, the intermediate being carried up with the base of the blade.

The cambial strands after their inception either below the insertion of the leaf, as in the earlier strands, or within the leaf rudiment, extend both upwards, higher into the leaf, and downwards into the axis. The upward differentiation of the majority of the strands extends into the blade either before or soon after the rapid elongation of the sheath. The marginal nerve (Fig. 2, 7 *m''*), however, does not develop upwards into the blade, for it differentiates in that part of the sheath which is continuous with the ligular ridge, and therefore develops upwards into the ligule.

The young blade, after the formation of the ligule, contains a mid-rib and six strands in each half of the lamina. The blade at this stage is traversed by six longitudinal furrows forming seven ridges; the mid-rib runs in the median ridge, and each of the other ridges usually contains two strands, a large and a small, though the disposition of the smaller strands is not quite regular. Any subsequent bundles which may be added to the complement of the blade are formed from cambial strands laid down in the blade independently of those in the sheath (Fig. 2, 7 and 8 *aaa*). Usually these additional bundles extend downwards to fuse with one or other of the six original blade traces. In large leaves some may continue their own course through the sheath into the axis (Fig. 2, 1 *a*). The positions of these bundles are not absolutely constant in all leaves; a typical arrangement in a mature leaf is shown in Fig. 3. The small bundles on each side of the mid-rib are constant, but there are slight differences in the distribution of the small bundles among the ridges of the leaf. Lateral expansion of the blade in large leaves is affected by additional furrows forming in the marginal part of the leaf, additional bundles also form in this region, and do not enter the sheath but fuse with the original nerves of the blade.

Meanwhile other marginal nerves develop in the sheath (*m'''*,

m''') which either extend upward into the ligule or remain, ending blindly in the sheath.

Transverse strands interconnecting the nerves of the sheath begin to develop soon after the formation of the ligule. These cross-connections can best be seen in young leaves dissected out under the binocular microscope. They form by tangential division of cells of the ground tissue between the intra-fascicular cambium of the longitudinal bundles, which have already become partly lignified. Such strands are sometimes more strongly developed between the distal

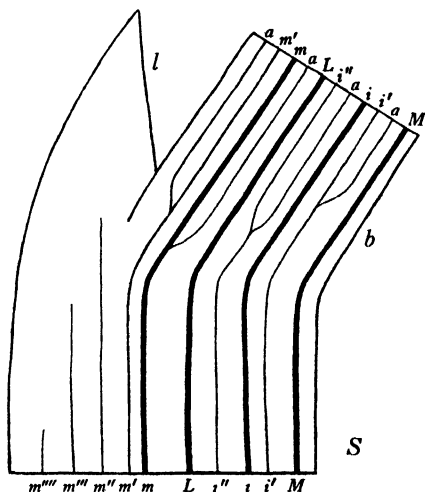


Fig. 3. *Deschampsia caespitosa* Diagram of the course of the vascular bundles in the left half of the adult leaf S, sheath; b, blade; l, ligule, other lettering as in Fig. 2

ends of the ligular nerves and those which enter the blade, giving the appearance that the marginal nerves have turned inwards to join the blade trace, sending up only a branch to the ligule. That this is not the case is shown by these transverse strands developing when the bundles which they connect are already lignified, that is they develop at the same time as the normal cross-connections.

MELICA ALTISSIMA L.

Stem apices of *Melica altissima* were prepared and sectioned in the same way as those of *Deschampsia*. An examination of the series of sections obtained shows that the development of the ligule in this species is in all essential details very similar to that in *Deschampsia caespitosa*. The margins of the sheath overlap slightly, but

remain united by a fold of tissue which prevents them from extending far around the stem apex. The base of the blade is broader in *Melica* (Fig. 1 C) than in *Deschampsia* so that the sheath cannot enter so extensively into the ligule, but the ligule forms precisely as it does in *Deschampsia*, and since the margins of the sheath are joined so are those of the ligule.

The development of the vascular tissue of the leaf is also very similar to that of *Deschampsia*. The broader base of the blade allows more of the marginal nerves to enter the blade, and the united margins of the sheath restrict the number that can enter the ligule. As in *Deschampsia* additional nerves form in the blade and fuse with the primary bundles before they enter the sheath, or in large leaves some may pursue an independent course through the sheath.

Up to the stage when there are five cambial strands on each side of the mid-rib of the young leaf, the development of *Melica* differs in no way from that of *Deschampsia*. The mid-rib and then the two laterals form within the stem apex and grow up into the crescentic rudiment. First a marginal and then an intermediate strand also have their origin in the stem apex, but the next pair on each side, another marginal and intermediate, originate within the rudiment itself. After this the marginal strands do not form in an outward succession as they do in *Deschampsia*; first one is laid down in each half of the leaf outside the laterals, the next is again to the outside but is in the median line of the cylindrical sheath opposite the mid-rib and fits into the series of both the right and left halves of the leaf. The next pair of marginal nerves is formed, one in each half of the leaf, just outside the first marginal nerve to be laid down. This last pair of marginal nerves may not reach the axis independently in some leaves but fuse with one or other of the adjacent strands (Fig. 4, 1 and 4). Another intermediate nerve is formed next to the lateral, as is the rule in *Deschampsia*.

There are now in each half of the young sheath a lateral, three intermediates, and four marginal nerves, and in the median plane a mid-rib and a marginal nerve. Of these all except the last enters the blade. The blade becomes very broad at an early stage, and before the sheath has completely encircled the stem apex the margins of the blade overlap (Fig. 4, 1 and 2). Within the blade many additional strands are formed, a typical disposition for an adult leaf being shown in the diagram (Fig. 5). There is very little difference between the arrangement in individual leaves; in some one of the additional nerves (usually that next the mid-rib) may be absent, or more

usually two or three extra nerves may be added either between the others or marginally. Usually the half of the leaf which encloses the

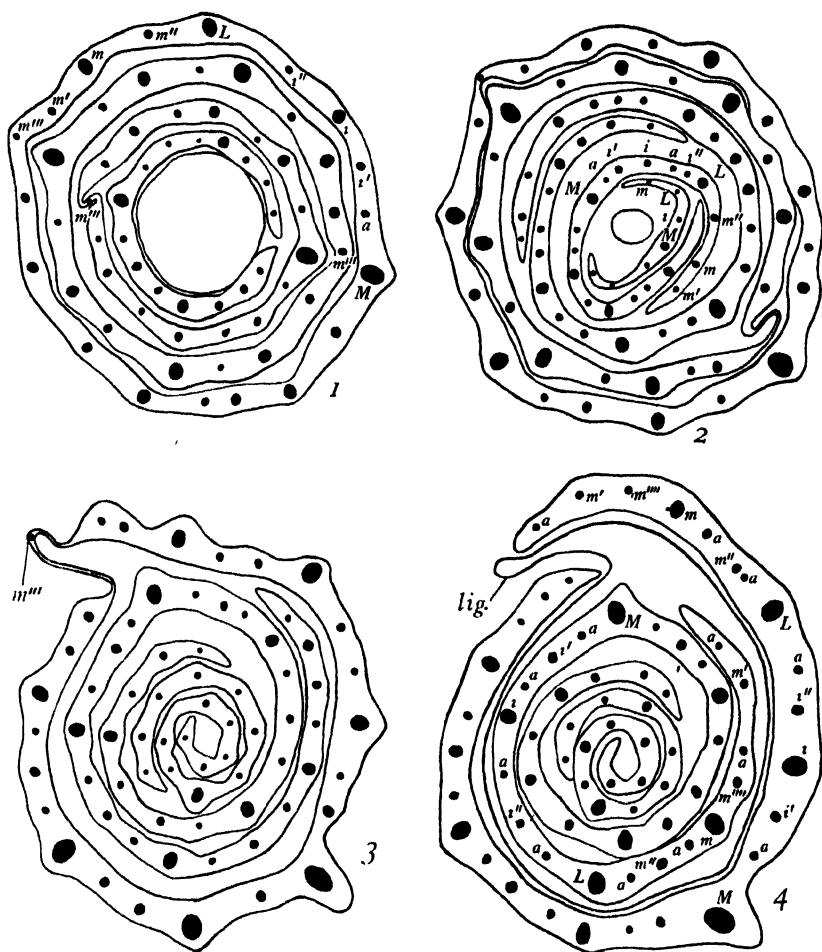


Fig. 4 *Melica altissima*. Transverse sections through the stem apex and young leaves, $\times 50$. Lettering as in Fig. 2.

Note. In the fourth oldest leaf the sheath is still open (1) but additional nerves have formed in the blade (2). In the third oldest leaf the ligular nerve (m''') has formed (1) but there is no trace of the ligule. The second oldest leaf has a ligule rudiment (not shown in these figures) and nearly all the bundles of a mature leaf.

other in the bud has one or two more nerves than the other half. The most important difference between *Deschampsia* and *Melica* is the earlier and greater development of the blade in the latter. In

Deschampsia none of the additional strands of the blade has formed before the ligule becomes apparent. In *Melica*, on the other hand, all but one or two of the additional nerves have formed before there is any sign of the ligule (Fig. 4, 2).

When the margins of the leaf rudiment encircle the stem they unite and the sheath becomes closed, as it does at the same stage in *Deschampsia*. The base remains cylindrical but the margins continue to grow as the sheath elongates, so that there is a slight overlap, the margins, however, remain united by a very thin fold of tissue in which the median marginal nerve runs (Fig. 4, 1 and 3). In large leaves a pair of very minute nerves may accompany this nerve and

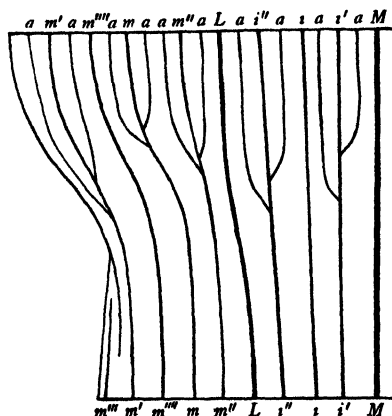


Fig. 5. *Melica altissima*. Diagram of the course of the vascular bundles in the left half of the adult leaf. Lettering as in Fig. 2.

fuse with it below. Growth does not continue long, so that there is only a slight overlap in the sheath. The ligule now forms as an upgrowth at the junction of blade and sheath, and as in *Deschampsia* the free upper border of the sheath is included, but unlike that species the extent of the upper border that is free is very limited owing to the broad leaf-base and the united margins of the sheath. In grasses with closed sheaths the ligule may either be a small crescent which does not include the upper border of the sheath, as, for example, in *Dactylis*, or it may be more extensive, a complete circle in the present instance, and even receive nerves from the sheath. In *Melica* the sheath enters into the ligule just as it does in *Deschampsia* and in *Phyllostachys* next to be described. The ligule appears to be a uniform upgrowth, but reasons for considering it to consist of two distinct

regions, a sheath region and a median region, will be advanced at the end of this paper.

Transverse cambial strands connecting the longitudinal bundles of the sheath begin to appear soon after the ligule rudiment appears. The first to form are between the more median nerves near the junction of the sheath and blade, and later they appear in a downward and an outward succession. The cambial cells which form these cross-connections are formed from cells of the ground tissue by tangential divisions. The cells which divide are in no apparent respect different from the rest of the ground tissue, but their contents become more deeply staining after the divisions have taken place. These transverse cambial strands form between the longitudinal bundles when the latter are fairly well advanced in their development, and some of their xylem elements have become lignified.

The formation of these cross-connections could be seen very clearly in this material, even in the very earliest stages. The tangential divisions of the cells of the ground tissue do not begin at the same time throughout the length of the young strand, but in all the youngest strands examined the cells next to the more marginal of the two longitudinal nerves which are to be interconnected divide first, and the cambial strand is formed from without inwards. This is also the case in the bamboo *Phyllostachys*, next to be described, and may be general in the Gramineae. The course of these cross-connections is often undulating owing to the irregular arrangement of the cells of the ground tissue from which they form.

In *Melica altissima* the ligular bundle is unconnected in its upper part to the longitudinal bundles next to it, and ends quite blindly in the ligule. In *Deschampsia caespitosa* the most marginal ligular nerves are frequently also unconnected, ending blindly, but the inner ligular nerves are always connected by well-developed cross nerves to the blade traces. In *Phyllostachys aurea* the ligular nerves are all connected in this way.

PHYLLOSTACHYS AUREA A. AND C. RIVIÈRE

Several apices of this species were sectioned and stained in the same way as those of *Deschampsia*. The early development of the leaf rudiments and their vascular bundles is not described, as this material with its small cells and large number of cambial strands is not suitable for showing those stages. The interpretation of the morphology of the ligule advanced by Colomb was based largely on the arrangement of the bundles in the adult bamboo. *Phyllostachys*

was chosen for this investigation of the development of these bundles because it shows his "stipular" arrangement very clearly. It was hoped by following the development of these nerves to obtain evidence for or against their stipular nature.

In *Phyllostachys*, as in most bamboos, the lamina of the leaf is well developed. This expansion of the blade begins very early, so that when the sheath is cylindrical the blade is already much rolled within the bud. At this stage an extensive vascular arc has been laid down consisting of a regular series of alternate large and small bundles. In all the specimens examined the sheath, at the stage

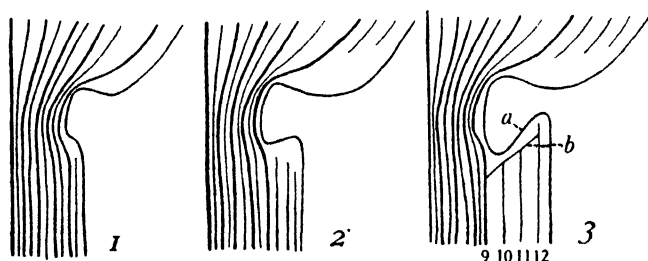


Fig. 6. *Phyllostachys aurea* Diagrams of the courses of the vascular bundles in the right half of the ligular region, at three stages in the development of the young leaf 1, the first indication of the free upper border of the sheath, with one bundle ending below it 2, the upper border of the sheath with three nerves ending below it. 3, the three nerves connected by a transverse nerve *a*, the free upper border of the sheath (the ridge or "talon"); *b*, transverse nerve *g*, most marginal nerve to enter the petiole 9, 10, 11, 12, nerves in the overlapping part of the sheath

when it was a simple cylinder, had the same number of bundles, viz. a mid-rib, and four large alternating with four small bundles in each half of the sheath. All these strands run up into the blade where they diverge widely in the lamina.

The sheath does not remain cylindrical but the margins continue to extend around the circumference, overlapping each other. At the same time the lamina becomes even broader, leaving a short petiolar region between the two. The next strand to be differentiated in the sheath does not extend upwards through the petiole into the blade, but remains ending blindly below the free upper border of the overlapping part of the sheath (Fig. 6, 1). It is soon succeeded by two more strands which also end blindly in the sheath (Fig. 6, 2), and in all the material examined no further marginal nerves are developed in the sheath.

In the blade many marginal nerves are developed as the lamina

expands, but these do not extend downwards into the sheath but eventually fuse with the marginal nerve of the petiole.

Soon after the first formation of the free upper border of the sheath with its first nerve, the ligule develops as a ridge of tissue on the ventral surface of the leaf at the junction of sheath and petiole. The ligule therefore develops at a later stage than in *Deschampsia*, where the upper border of the sheath is never free but as it develops it is incorporated in the ligule. The result is that the crescentic insertion of the ligule extends on to the overlapping margins of the sheath, leaving the upper borders free as the ridge or "talon" of Colomb in the adult ligule.

Transverse cross-connecting strands form between the nerves of the sheath throughout its length. Cross-connections appear successively between the 9th and 10th, 10th and 11th, and 11th and 12th longitudinal nerves of the sheath, at or near their distal ends, the complete transverse nerve running along the free upper border of the sheath, above the insertion of the median part of the ligule (Fig. 6, 3). It was this nerve that Colomb considered to be a stipular branch of the foliar bundle, from which he concluded that the ridge or "talon" was of the nature of a stipule. The development of the ligule has shown this ridge to be the part of the overlapping sheath which is above the insertion of the median part of the ligule. The transverse bundle (*b*) cannot be a stipular branch of the longitudinal bundle (*g*), because the cross-connections in *Phyllostachys*, as in *Melica*, develop from the more marginal nerves inwards; the transverse nerve therefore develops in three distinct sections.

DISCUSSION

Most botanists have considered the ligule to consist of fused stipules. If stipules be defined as lateral appendages of a leaf at its insertion on the axis, the ligule could not be stipular unless it is assumed that the sheath consists of a petiole fused to a pair of stipules except at their tips which unite to form the ligule. There is no evidence to be found in the development of the sheath which indicates that it is a composite structure, and in the Glumiflorae, at least, the regular development of the vascular arc (Bugnon, 1921; Guichard, 1929) tells very strongly against such a supposition. If, however, the term stipule be admitted for projections of the top of the sheath a ligule formed by their fusion might be considered stipular (Arber, 1925), but it is difficult to see how this could take place without the formation of a median upgrowth between the two pro-

jections. This seems to be the only sense in which the ligule, or rather its lateral parts, may be considered stipular; but there seems to be no justification for this use of the term stipule.

The conclusions arrived at by Colomb as to the triple nature of the ligule have already been set out in an introductory paragraph, and discussed to some extent after the development of the ligule in *Phyllostachys* had been described. It seems impossible in the light of the developmental evidence to separate his "talon" from his sheath region, as they both form as part of the sheath and become separated merely by the line of insertion of the median part of the ligule. Still less can this "talon" be considered stipular, as the development of its transverse nerve shows it to consist of a series of cross-connections, and in no way to be a branch of a foliar nerve. In a great many bamboos this transverse nerve runs for a part of its course outside the "talon" and within the sheath region, showing their essential unity. It would seem necessary to reduce Colomb's three regions of the ligule to two, viz. the paired sheath regions and the median upgrowth between them.

Bugnon (1921, p. 57) concludes that the primitive condition of the sheath in grasses is closed, and that the ligule in such grasses is a purely accessory structure. In grasses with a spiral insertion the overlapping margins of the sheath are able to take part in the formation of the ligule, and may become nerved. In grasses with the sheath closed, the difference in circumference between the blade base and the top of the sheath leaves free that part of the upper border of the sheath opposite the mid-rib. It would be possible even in such completely closed sheaths for the ligule to include the free upper border of the sheath. *Melica altissima* and *M. uniflora* have not only tubular sheaths but tubular ligules, and in the former a median nerve opposite the mid-rib runs from the sheath into the ligule. The leaves of the genus *Carex* show a close parallelism in both anatomy and development to those of the grasses (Guichard, 1929, p. 111), and here the typical closed sheath shows clearly a free upper border which is usually membranaceous and nerveless but which in *C. palustris* is traversed by a median nerve. In the Liliaceae Hoffman (1933) describes nerves ending blindly beneath the free upper border of the closed sheath of *Allium cepa*.

Thus there seems every reason to suppose that even in a closed sheath part of its upper border, with or without vascular bundles, may enter into the formation of the ligule. The increase in importance of the sheath in leaves with a spiral insertion would be one of degree

rather than of kind. In *Deschampsia* and *Melica* the first ligular nerves at least develop in the regular sequence of the vascular arc, and cannot be said to be behindhand or accessory as Bugnon suggested. The comparatively early stage in the development of the vascular arc at which the first ligular strand is laid down in *Deschampsia*, as in *Melica*, supports the view that possibly part of the primitive closed sheath is included in the ligule of the former, as well as the overlapping margins of the split sheath.

In *Deschampsia* the marginal and median parts of the ligule develop so simultaneously that it might be thought that they are not distinct but a uniform upgrowth of the top of the sheath. Other considerations, however, tend to show that these two regions are independent of each other and quite distinct in origin. In the first place in many grasses the median part of the ligule is absent, when the upper border of the sheath may remain as more or less prominent auricles. This is well seen in many bamboos where closely related forms may either have a prominent ligule as in *Phyllostachys aurea* (Fig. 1 B) or the sheath may end in a projecting lobe on each side of the petiole. In many grasses, particularly in tropical genera such as *Echinochloa*, *Sporobolus*, and *Eragrostis*, the ligule is reduced to a line of hairs and frequently the sheath has a pair of small auricles at its upper end. The development of these lobes of the sheath in *Phyllostachys* shows them to develop as part of the sheath which is considerably broader than the petiole and that they later become joined by the upgrowth of the median part of the ligule.

The view that the ligule is composed of two quite distinct parts is further supported by a study of the transitional structures between the lemma and the normal vegetative leaf in proliferating spikelets (Philipson, 1934). In *Deschampsia caespitosa*, where the ligule receives nerves from the sheath (Fig. 1 A), the lateral lobes of the lemma with their nerves are seen to become united by a median membranaceous scale. This scale in some of the intermediate structures between the lemma and the leaf is free from the lateral lobes, which then appear as lobes of the sheath. Similar scales are present on the adaxial surface of the lemmas of proliferating spikelets of *Poa alpina* where they form all but the extreme margins of the ligule, and of *Dactylis glomerata*, where they form the complete ligule. The relative importance of the lemma lobes in the formation of the ligule in abnormal lemmas corresponds with that of the sheath in the ligule of the vegetative leaves of the same species. The membranaceous scale corresponds to the median part of the ligule, and the

lateral lobes of the lemma to the sheath region. The median part of the lemma is very rarely nerved, but in some bamboos and in *Oryza sativa* it receives ventral branches of the foliar bundles.

In conclusion the ligule may be said to consist of the free upper border of the sheath, which is usually split, and a median upgrowth of the adaxial epidermis of the leaf. Both these regions may be absent, singly or together, but in the absence of the latter the grass is considered non-ligulate.

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SEED SETTING AND FLOWERING IN *BRACHY-
PODIUM PINNATUM* BEAUV., *B. SYLVATICUM*
R. AND S., *DESMAZERIA SICULA* DUM. AND
LAMARCKIA AUREA MOENCH.

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INTEREST in the Gramineae has steadily advanced within the last fifteen or twenty years. This revival has in no small measure been due to the realisation of the relative value of the various species in agricultural practice and especially of the importance of indigenous strains. Development has, therefore, followed along two interdependent lines: (a) improved management of the sward, and (b) improvement of the individual constituents making up the sward. It was early discovered that advance in the latter direction presupposed a knowledge of the floral mechanisms of each species to be improved.

Considerable attention has, therefore, been given to this aspect of the work in various parts of the world. Of the numerous papers which have been published we may mention those by; Evans (1916), Frandsen (1917), Fruwirth (1916), Splechtner (1922), Jenkin (1924), Kirk (1927), Gregor (1928), Troll (1930), and Kramer (1932). These workers have been concerned chiefly with those grasses most used agriculturally in their respective countries.

The economic value of any grass species, however, is relative, its use being more or less controlled by such factors as soil fertility, availability of water, and their modification on account of latitude (and/or altitude). An anonymous writer in *Nature* (1930) sums up the situation admirably when he says "in regard to grasses no preconceived reservation should be made as to what species might prove valuable; thus for certain difficult areas in New South Wales, such an unlikely grass as tor grass (*Brachypodium pinnatum*), which is nothing but an objectionable weed in Britain, has been shown to be of possible value and worthy of more serious trial; . . .".

In order, therefore, to widen the range of our knowledge of the grasses, investigations into their seed-producing capacities and flowering habits were carried out at the Welsh Plant Breeding Station. In all, twenty-nine perennial and thirty-five annual species

have been reported upon (Beddows (1931 *a, b*), Jenkin (1931 *b, c, d, e, f*), and Stapledon (1931)).

The present paper deals along similar lines with four further species, two of which are perennial and two annual, and it is, therefore, complementary to those quoted above.

The plants used were maintained in earthenware pots of suitable size which were brought into a large greenhouse just prior to the commencement of flowering.

The selfing capacities of the plants were tested by enclosing one or more inflorescences within pollen-proof bags, a technique fully dealt with by Jenkin (1931 *a*).

BRACHYPODIUM PINNATUM BEAUV.

Tor grass is a perennial species with a slightly creeping habit found chiefly in open downs on calcareous soils. Its awns are shorter than the inferior palea (quarter to half as long).

The plants available were derived from two sources, Czechoslovakia and the Cotswolds. The latter gave fewer seeds when selfed although both lots were fairly similar morphologically. The results are summarised in Table I, where the yields from free-flowering panicles are given for comparison.

TABLE I

Seed-setting in enclosed and free panicles of B. pinnatum.

Greenhouse, 1930-4

Station No and origin	No. of plants	No of isola- tions	No of pani- cles	Enclosed		Free			
				Caryopses per		Caryopses per			
				Panicle	100 spike- lets (range)	No of samples	No of pani- cles	Panicle	100 spike- lets
Bs 505 Czecho- slovakia	4	41	240	4.2	58.3 (0.0-295.0)	1	6	21.8	327.5
Bs 1093 Cotswolds (England)	23	52	382	1.4	10.9 (1.0-120.0)	18	87	30.1	382.5

The data in Table I show that *B. pinnatum* is normally a cross-fertilising species, and that self-fertilisation, though possible, is low.¹

¹ Less than three caryopses per spikelet were obtained from the most effective case of self-fertilisation encountered, and this cannot be regarded as high in view of the fact that spikelets may possess from 7 to 18 florets (average 11 from 480 spikelets). From open pollination the maximum yield was 7.4 caryopses per spikelet.

The contention of Körnicke (1890) that it is fully sterile has not been confirmed.

The wide range in the number of seeds obtained from selfing is not to be regarded as something unusual. In his paper on self-fertility in *Lolium perenne*, Jenkin (1931*b*, p. 101) shows how variable the yields may be for any one plant, and that it is necessary to conduct a large number of trials with each individual before the limits of its potential selfing capacity can be ascertained.

Ascherson and Graebner (1901, p. 632) state that *Brachypodium pinnatum* flowers but sparingly under the conditions obtaining when it overruns large areas in woods and parks. In this connection it has been observed that plants kept for any length of time in pots produce relatively few flowering heads. This is considered to be due (*a*) to the creeping habit of the plant (cf. *Agropyrum repens*, Beddows, 1931*b*, p. 65), and (*b*) to impoverished soil conditions. Plants in good condition produce numerous panicles.

Flowering. Information regarding the mode of flowering in Tor grass is scanty. Warnstorff (1896) states that both the stigmas and anthers ripen simultaneously, the florets opening before 6 a.m. and shedding their pollen between 6 and 7 a.m. He adds that the stigmas are widely exserted and the anthers pendulous on long filaments, so that autogamy is not possible. Körnicke (1890) gives 6 a.m. as the period of maximum pollen liberation. Kirchner, according to Knuth (1909), reports that the stigmas remain exserted after the anthers have fallen.

The observations on flowering made at Aberystwyth and detailed below are in general agreement with those of earlier workers. Flowering occurred from about the middle of June to early July. On any particular day the florets may begin to open as early as 4 a.m. (G.M.T.) but the zenith of pollen liberation usually falls between 5 and 6 a.m., while occasional florets may dehisce their anthers as late as 12 noon. The stigmas, which are exposed before the anthers, are exserted either simultaneously or within a short interval of one another. In the early stages of flower opening the stigmas are retained more or less erect in between the bunched anthers. As a result of the gradual widening of the palcae and the elongation of the filaments the anthers disengage and separate, so that ultimately the stigmas are released and spread out one to each side of the floret. The stigmas are large, the exposed portion being about one-fourth of the inferior palea in length. They may extend at right angles to the edge of the floret or bend downwards towards its base. One

bifurcated stigma was observed which combined both types. The anther filaments remain taut until the anthers are carried to the mouth of the now open paleae, where as a result of further elongation and the weight of the pollen sacs they gradually lean over. At, or soon after this stage, the anthers, which are 5-5.5 mm. long, begin to split, a pore appearing on each side just behind the distal end. The pollen, however, seems to be held within the sac until more or less pendulous, but any disturbance of the panicle will cause the pollen to be liberated in irregular spurts from each pore. The anthers do not leave the floret together, but in sequence, which also holds for dehiscence, showing that they do not ripen quite simultaneously.

The times taken over the phases of flowering were noted in a few cases. The complete opening of the paleae occupied about 50 min., exertion of both stigmas 3-4 min., while the period from the first loosening of the anthers to their dehiscence took about 25 min. In general the process of flowering in *Brachypodium pinnatum* is quite leisurely. The anthers may remain attached by their filaments for 6 hours or longer. The paleae, even in florets effectively pollinated, do not close up again for a considerable period, 8-10 hours, or in some cases 12 hours. The stigmas lose their fresh appearance after fertilisation. They do not readily disintegrate, but remain, for many days, as a white "stripe" along each side of the long spikelets, acting as a conspicuous indicator of the progress of flowering.

The evidence obtained from the study of the mode of flowering confirms Warnstorff's conclusion that normally autogamy¹ is improbable and that the seed formed on selfing the panicles is the result of geitonogamy. In nature the seed produced will be due mainly to cross-fertilisation (xenogamy).

Anther colour varies from plant to plant, but within each plant it appears to be constant and uniform. The gradations in colour, due to the presence of anthocyanin of varying intensity and distribution, so noticeable in *Lolium perenne* and *Festuca pratensis*, were not encountered in *Brachypodium pinnatum*. The difficulty of classifying the colours was overcome by comparing fresh unburst anthers with the colour charts given in *Répertoire de couleurs* of Dauthenay (1905). The shades noted are named in Table II.

¹ Autogamy fertilisation of a flower by its own pollen. Geitonogamy: fertilisation between neighbouring flowers on the same plant. Xenogamy: cross-fertilisation between sexual elements borne by different plants of the same species. (See Jackson, B. D., *A Glossary of Botanical Terms*, 4th ed. 1927, London; and Rendle, A. B., *The Classification of the Flowering Plants*, 1, 2nd ed., 1930, Cambridge.)

TABLE II

Anther colours observed in certain plants of B. pinnatum. The numerals (24 (1), etc.) refer to the colours and shades in Répertoire de couleurs. 28. vi. 1934; 5.30 a.m. G.M.T. Greenhouse

Plant No.	Anther colour	Colour and shade number	Remarks
Bs 505 (8)	Yolk yellow	24 (1)	24 (1) but with tinge of green
" (11)	Yellow-green	16 (1)	But edges and especially the tips more definitely green*
Bs 1093 (11)	Amber yellow	28 (2)	Burst anthers 28 (3) and (4)
" (14)	"	28 (2)	
" (15)	"	28 (2)	
" (18)	"	28 (3)	
" (23)	"	28 (4)	
" (19)	Honey yellow	35 (1)	
" (17)	Apricot	53 (1)	Burst anthers 53 (2)

* Green colour in anthers is often associated with male sterility (see below), but those of Bs 505 (11) were noted to contain pollen. It should be stated that this plant is irregular in respect of pollen production. Selfed, it has given 68.2 seeds per 100 spikelets (range 0-132.9) in 11 isolations. In the case of plant Bs 505 (13) the anthers were shorter and narrower and of a markedly green tinge. These anthers showed not the least tendency to dehisce. The plant was, therefore, completely male sterile.

Anthers which had burst were generally a shade or so deeper than when in the fresh condition. It will be observed that none of the plants available gave pale brick-red anthers, the colour given by Ascherson and Graebner (*loc. cit.*).

One of the florets examined contained four anthers, all of which were normal.

BRACHYPODIUM SYLVATICUM, ROEM ET SCHULT

Wood false brome is a shade-loving, caespitose perennial. The awns are as long as, or longer than, the inferior palea (up to $1\frac{1}{4}$ as long).

Six plants only were available and the data obtained from them are given in Table III. It will be seen that the five plants Bs 1072-1076 were all fairly highly self-fertile—an average of 4.7 seeds per spikelet with a range of 2.4-8.8. This is approximately 60 per cent., since the spikelets had an average of eight florets each.¹ Körnicke (*loc. cit.*) stated that *B. sylvaticum* was "fully fertile". An interesting exception is furnished by Bs 1071. In this plant the yield is but 9

¹ The average of 276 spikelets. The range was 6-14 florets per spikelet.

per 100 spikelets—a result more in keeping with those for *B. pinatum* (q.v.). Bs 1071, however, appears to be in every way typical of *B. sylvaticum*.

TABLE III

Seed-setting in enclosed and free panicles of B. sylvaticum.
Greenhouse, 1933-4

Station No.	Enclosed					Free				
	No. of plants	No. of isolations	No. of panicles	Panicle	Caryopses per	No. of samples	No. of panicles	Panicle	Caryopses per	100 spikelets (maximum)
					100 spikelets (range)				100 spikelets (maximum)	
Bs 1071	4	15	123	0.8	9.2 (0.41.9)	5	19	20.3	281.7 (583.8)	
Bs 1072-1076	18	52	465	39.2	470.1 (242.9-881.8)	26	115	45.4	610.4 (908.8)	

Flowering. The flowering habits of *B. sylvaticum* do not appear to have been described.

The information given below is based upon observations made during mid-July at Aberystwyth on plants in pots and in their natural habitat.

Investigating the time at which the florets opened provided the writer with both stimulation and exhilaration, for in spite of rising increasingly earlier on several consecutive days the onset of flowering was not encountered. Thus even arriving at the greenhouse at 2.20 a.m. (G.M.T.) and at Cwm Lane¹ at 2.35 a.m. proved to be too late. Watching the plants during the late evening also proved fruitless, since nothing happened until after dusk. One floret, however, was observed at 10 p.m. in the greenhouse to have opened its paleae and to be exerting its anthers. No other cases were seen, partly no doubt because the plants had practically finished flowering, and this made the search for odd panicles in flower, by the light from an electric torch, rather a matter of chance. Suitable panicles from wild plants were then cut and kept in water in the bedroom. At 10.55 p.m. five florets were seen to be opening their paleae, and by 1 a.m. at least a dozen florets were open, their anthers exerted and splitting. A plant, which had but recently started to flower, was also dug up with a good ball of soil, potted, and kept under observation in the

¹ Cwm Lane is a rough sunken roadway with a larch plantation on one side and a mixed woodland of oak, ash and hazel on the other. The latter near the roadway has a ground flora of *Brachypodium sylvaticum* (dominant), *Dactylis glomerata*, *Aira caespitosa*, fern and bramble

bedroom. The first night no flowering occurred. The next night at 2.15 a.m. a floret had exerted its anthers, and at 4.30 a.m. at least half a dozen had done so. The third night at 2.45 a.m. two florets were opening their paleae, and by 6 a.m. six florets had their anthers pendulous. It may, therefore, be assumed with fair certainty that flowering in *B. sylvaticum* occurs between dusk and dawn, most probably between 11 p.m. and 2 a.m.

The weather during the two periods of flower observation at Cwm Lane may be summarised thus: the days between July 6th and 10th were sunny and hot (11.7–15 hours' sunshine, average 14.0; maximum temperatures 80°–84° F., minimum 59°–63° F.), those between July 16th and 20th cooler, rather overcast with some rain (1.0–7.5 hours' sunshine, average 4.1; maximum temperature 62°–75° F., minimum 56°–59° F.). The conditions had thus altered considerably, but the change was not such as to interfere with flowering, which continued as usual during each night. May it not be that flowering as it does at night in a more or less sheltered habitat *B. sylvaticum* is not affected by the changes in meteorological conditions ordinarily obtaining in July?

The mode of flowering was, in general, similar to that in *B. pinnatum*. The stigmas may be normally exerted and the anthers become pendulous before dehiscing, but failure to accomplish these acts completely was not infrequent in stigmas and/or anthers. The long awns and the stiff glumes did not prevent normal exertion. In the case of the stigmas failure to exert one or both was found to be largely decided by their arrangement within the inferior palea. Dissection of ripe but unopened florets showed the stigmatic plumes to be bent over, so that their edge and tips were tucked into the overlap of the larger palea. They were, therefore, often unaffected by separation of the paleae and remained unexposed.

The anthers also acted irregularly, so that one or more might be seen to split within the floret, or one or more might begin to dehisce inside and then be exerted. All combinations of anther and stigma behaviour could be found on one and the same panicle.

The angle to which the paleae separated did not seem a deciding factor, since florets showing normal exertion opened no wider than the others.

Fresh anthers measured from 3.5 to 4.5 mm. in length. Ascherson and Graebner (1901, p. 635) give their colour as yellowish; the above were classified as canary yellow (17 (1) of Dauthenay).

Self-fertilisation by autogamy and geitonogamy is possible in

B. sylvaticum. In the open cross-fertilisation might also take place, at least to some extent.

DESMAZERIA SICULA DUM.

Desmazeria sicula is indigenous to the Mediterranean region, especially Sicily and Malta. It is cultivated to some extent for ornament and frequently used for edging (Hitchcock, 1914). In habit it is strictly annual, and seed sown in boxes on April 25th, May 13th and June 4th had developed into mature plants starting to flower in 71, 67 and 56 days respectively.

Self-fertility was low in *D. sicula*—thus 120 original or extracted plants from Czechoslovakian seed, in 124 isolations during 1930-4, gave an average yield of only 2.1 seed per inflorescence, or 13.3 per 100 spikelets (range 0-269).¹ The data include 57 instances of isolations which failed to set seed, although the plants were male-fertile, but do not include those from male-sterile plants whose anthers did not dehisce and therefore could not be selfed. The free-flowering inflorescences of all plants readily set seed, averaging 76 per head or 508.9 per 100 spikelets (maximum 1208.1).

Flowering. Flowering takes place in the morning between 5 o'clock and noon G.M.T. It commences in the lowest florets of the uppermost spikelets, but soon spreads to all the spikelets.

The effects caused by meteorological conditions on flowering in *D. sicula* are not clear. This is due in part to the fact that the data available (Table IV) cover too short a period; and that the plants matured out of their normal season,² which is May or June when the days are longest.

It will be noticed that although sunshine following dull days was effective in bringing about anthesis, the continuance of sunny conditions did not give more than one day in flower.

In the individual floret the onset of flowering is shown by the opening of the paleae, which ultimately make an angle of about 30°, or sometimes even 45°. This process is apparently initiated by the

¹ In such relatively high yields contamination is always to be suspected. The present instances, however, may have been due to inherent self-fertility, since the spikes were noted as "reliable", i.e. had not flowered up to the time of bagging. Wide variations in capacity for setting of cross-fertilised grasses are not unknown (see Jenkin, 1931b) *D. sicula* being an annual, a check by re-seeding is impossible.

² The seeds, from which these plants developed, were purposely sown late (June 4th) in order to avoid the flowering season of those economic species which are the chief concern of the Station.

emerging anthers. The stigmas are at first erect and hidden within the grouped anthers,¹ but later when the paleae have opened more widely and the filaments elongated somewhat the anthers separate. The stigmas are then released and spread out to each side of the floret. The exposed portion of the stigmas measures from quarter to about half the length of the inferior palea. The anthers at this stage may still be within the paleae but usually they are well up towards the apex of the floret. The stigmas are not always exerted. They are sometimes caught and held by the sides of the rather deeply bowed outer palea during the process of spreading out preparatory to exertion. The anthers generally shed their pollen when pendulous but dehiscence can begin, and may on occasion even be completed, while they are held more or less erect at the mouth of the floret. It is, therefore, possible for pollen to fall on to the stigmas of the same flower, or at least within its own paleae or on those of the florets below.

TABLE IV

General weather conditions and the incidence of flowering in D. sicula plants in boxes—Gardens, 1934. (The meteorological data were recorded at the Plant Breeding Station farm—altitude of farm meteorological station is 452 ft.; that of gardens 120 ft.)

Date	Weather conditions at gardens	Temperature		Sun- shine hours	Rain in.	Condition of inflorescences
		Max.	Min.			
July 28	—	65	57	2.5	0	—
" 29	A.m. very dull and wet; p.m. dull	63	58	0	0.67	Not in flower
" 30	Bright intervals, rather close	68	58	3.2	0.03	"
" 31	Cool in early morning, sunshine	66	57	10.4	0.03	Flowering
Aug. 1	Wet and cool	62	54	0.6	0.53	Not in flower
" 2	Wet and cool	58	55	0	0.30	"
" 3	Bright and warmer	61	54	9.3	0	"
" 4	Cool, bright with heavy dew	62	57	10.1	0.02	Flowering
" 5	Dull, dull and showery, then very wet	63	51	0.5	0.52	Not in flower
" 6	Dull, showery, then with bright intervals	67	56	6.7	0.02	"
" 7	Dull, showers, bright intervals	68	57	1.0	0.07	Flowering
" 8	Warm, bright with dull periods, showers	68	52	8.4	0.13	Not in flower
" 9	Overcast and windy	62	56	3.2	0.44	Flowering

The low seed yields from selfing demonstrate that "own" pollen is not very effective and that geitonogamy does not readily occur. The unprotected inflorescences on the other hand set seed in abund-

¹ If, during emasculation, care is not taken the stigmas are either broken or pulled out with the anthers.

ance when pollen was available; the more copious the pollen supply the higher the percentage of seed set. Under natural conditions cross-fertilisation (xenogamy) would be almost exclusively operative.

LAMARCKIA AUREA MOENCH.

Lamarckia aurea is an annual originating from the Mediterranean region, but now a widely spread ruderal species. Its spread may not be unconnected with the clinging tendency of the fascicles and with the fact that the seeds are not readily dislodged. In Britain it has been recorded on the Tweedside (Hayward and Druce, 1919). It has become naturalised in southern California and is known as golden-top (Hitchcock, 1920).

High self-fertility is a very marked feature of *L. aurea*; 43 isolations on 39 plants gave an average of 82 seeds per 100 fascicles. This approximates fairly closely to the 89 given by the exposed inflorescences (cf. Table V). It will be noted, however, that there are

TABLE V
Seed-setting in enclosed and free panicles of Lamarckia aurea.
Greenhouse, 1930, 1931 and 1934

Station No and origin	Enclosed					Free				
	No of plants	No of isola- tions	No of pani- cles	Caryopses per		No of sam- ples	No. of pani- cles	Caryopses per		100 fascicles
				Panicle	100 fascicles (range)			Panicle	100 fascicles	
Bs 514 Czechoslovakia	21	21	42	79.8	80.5 (53.7-97.2)	9	22	95.1	83.3	
Bs 1178 Algiers	14	18	24	91.2	89.3 (42.0-175.9)	10	11	118.2	107.7	
Bs 1194 California	4	4	4	94.0	66.5 (55.0-85.2)	2	4	119.3	70.6	
Total	39	43	70	84.5	82.4 (42.0-175.9)	21	37	104.5	88.7	

some plants with low and others with very high yields. The former are caused by (a) the partial or complete failure of the panicles to emerge from their sheaths,¹ and (b) the presence of numerous vestigial fascicles in some of the inflorescences. In addition most panicles show a variable number, usually small, of apparently quite normal florets in the "fertile-spikelets" which fail to develop a caryopsis.

¹ The panicles must be enclosed at an early stage of emergence, as flowering often begins soon after they appear through the sheaths. Bagging of these young, delicate and generally short shoots requires more than ordinary care if normal growth is to result.

The unexpectedly high yields were caused by the appearance of fascicles containing two or more caryopses. These are considered in greater detail elsewhere. The only other record of fascicles with more than one seed is that by Arber (1928), who describes the presence of two per fascicle in material grown at Cambridge.

Flowering. Under natural conditions, *L. aurea* flowers from March to June (Ascherson and Graebner, 1901), April to May (Gren. et God. 1856). The opening of the florets takes place chiefly during the early morning; the time ranges from just before 4 a.m. to midday (G.M.T.). The actual daily period seems to be controlled by external conditions, thus:

June 26th. Flowering apparently over by 7 a.m.

June 27th. Continued from about 5 a.m. to 12 noon, when an occasional floret could still be found exerting its anthers.

June 28th. By 5 a.m. most of the anthers had already burst, and no further florets were seen to open although examined at intervals up to 9.30 a.m.

The stigmas emerge through the gap between the tips of the slightly separated paleae. The anthers, which are one-fourth the length of the inferior palea, come into view later. They begin to split and may even completely dehisce before becoming clear of the paleae; this process occupies about 15 min. The two antler-like stigmatic arms project from one to three times the length of the anthers beyond the apex of the floret. Since the majority of the fascicles tend to hang downwards, and, further, the anthers keep close together or separate only slightly, some of their pollen must, therefore, fall on to that portion of the floret's own stigmas projecting between and beyond them. Confirmation of this was obtained by microscopic examination, pollen grains being plentiful on the portion of the stigmas beyond the anthers but not on that nearer the floret.

The mode of flowering suggests that compulsory self-fertilisation (autogamy) is the rule in probably the majority of the florets. The close agreement in the seed data from protected and free panicles, together with the protective character of the sterile spikelets, suggests that cross-pollination is not likely to be effected. On the other hand, it must be remembered that the florets are proterogynous, so that cross-fertilisation is not ruled out.

The colour of freshly exerted anthers is amber yellow (28 (1)—Dauthenay) and yellowish-green when empty.

SUMMARY

The self-fertility of four species was tested by enclosing inflorescences in pollen-proof covers. The results thus obtained are compared with those from unprotected panicles.

Seed-setting capacity and mode of flowering are correlated for each of the species.

Brachypodium pinnatum Beauv. flowers chasmogamously and is highly self-sterile. Cross-fertilisation (xenogamy) is normally necessary for seed formation.

Flowering takes place during June and July between 4 a.m. and noon (G.M.T.).

Brachypodium sylvaticum R. and S. is also chasmogamous but self-fertility is high. Fertilisation usually occurs geitonogamously (i.e. by means of the plant's own pollen), but it can also take place autogamously (flore's own pollen), since one or more anthers may dehisce within the paleae. Under natural conditions cross-fertilisation might also be possible.

The plants flower in July, between dark and dawn.

Desmazeria sicula Dum. is an annual of the Mediterranean region. The florets are chasmogamous, normally cross-fertilised, and highly self-sterile. *D. sicula* is, therefore, a further exception to the rule that annuals are self-fertile. It flowers in May and June, or even in August, if the seed is not sown until June. The florets open between 5 a.m. and noon.

Lamarckia aurea Moench. is also a Mediterranean annual, but in contrast to *Desmazeria sicula* it is a widely spread ruderal species. The florets are proterogynous, and only slightly chasmogamous; but owing to the relative positions of anthers and stigmas at the apex of the floret, and the effectiveness of "own" pollen, autogamous fertilisation is probably general. It is, therefore, very highly self-fertile, the enclosed panicles giving practically as many seeds as those left unprotected.

Lamarckia aurea flowers from March to June, and during the latter month between 5 a.m. and noon.

The normal number of spikelets per fascicle is four, but exceptional plants with up to seven spikelets per fascicle have been found giving two or more caryopses per fascicle; the usual number is one.

The fascicles may therefore be considerably more complex than has hitherto been recognised.

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A FINAL LINK BETWEEN THE TERTIARY FLORAS OF ASIA AND EUROPE

(CONTRIBUTION TO THE AGE OF THE ARCTO-TERTIARY
FLORAS OF THE NORTHERN HOLARCTIC)

By A. KRYSH'TOFOVICH (LENINGRAD)

SEVERAL years ago I gave a sketch of the evolution of the Tertiary flora in Asia and of former phytographic regions(1), as well as of the changes which have taken place in the composition of the Tertiary floras of the Holarctic(2). In these articles I have endeavoured to prove the theory that in Asia and in north-west America, contrary to what happened in Europe and in south-east America, the flora took on the Tertiary aspect and systematic composition during the last stages of the Cretaceous. This fact has led even experienced palaeobotanists to wrong conclusions in regard to the age of some later Cretaceous and early Tertiary floras of Asia and north-west America. The next point in my papers was, that during the earlier stages of the Tertiary two different floras existed: one an evergreen Poltavian flora characteristic of the European Palaeogene, the other a temperate deciduous Turgayan flora which was developed at an early period (Late Cretaceous) in Asia, and possibly in the Arctic; which flora, moving westward and south-westward, reached Europe just at the Miocene period, at which time it became dominant even in this country, formerly the home of the Poltavian flora.

The stability of the temperate Turgayan flora in Asia during the Tertiary has made it impossible, or at least very difficult, to discriminate the different stages of the flora and the corresponding continental strata of the Tertiary in Asia, while the quickly changing Poltavian flora of Europe has shown successive phases of different composition during rather short periods. This has resulted in incommensurability of the Tertiary floras of Asia and Europe.

The Turgayan flora, as it moved to the west and south-west, changed but slightly in its composition and morphology, producing the impression of a very stable complex unfitted for the determination of short geological epochs. In but very few cases within the confines of Asia were other means met with for determining the age except the flora itself. In one case in the Aralo-Caspian plain remains of *Baluchitherium* were found in depressions of the Oligocene platform,

the beds of which are associated with *Ostrea prona*, *Pecten priscus*, *Nucula* sp., etc. The flora of the usual Turgayan composition was found together with, or a little above, the beds containing mammalian bones supposed to be Upper Oligocene. In a few cases the flora was associated with *Corbula helmersenii*, being considered as Upper Oligocene. According to the most reliable discoveries the Turgayan flora from its most typical localities in the horizontal beds of the Aralo-Caspian region has the following composition: *Salvinia Reussii*, *S. mildcana*, *Sequoia Langsdorfii*, *Taxodium distichum miocacnum*, *Phragmites oeningensis*, *Poacites* sp., *Populus mutabilis*, *Comptonia dryandroides*, *Juglans acuminata*, *Carpinus grandis*, *Corylus insignis*, *C. Macquarrii*, *Alnus nostratum*, *Fagus Antipovii*, *Quercus Gmelinii*, *Q. Nimrodi*, *Q. Drymeia*, *Q. Alexeievii*, *Ficus populina* (?), *Liquidambar europacum*, *Zizyphus tiliacfolius*, *Carpenterianthus turganicus*.

Nevertheless, although I was myself convinced of the antiquity of the Turgayan flora, I considered it as not sufficiently proved, and have constantly looked for reliable evidence. The position was rendered more difficult by the great spatial gap between the two regions inhabited by the same temperate flora: namely the Palaeogene Asiatic region and the Neogene European region, owing to which fact it was impossible to disclose the true succession of these beds even upon another basis. Until lately all known Asiatic floras were situated far beyond the Urals, whilst in European Russia the Poltavian evergreen floras were not known beyond the Volga, and the temperate Sarmatian (Upper Miocene) floras were known only on the shores of the Sea of Azov. While the Poltavian floras (Palaeocene and Eocene; and in the West, Lower Poltavian) are purely tropical or strictly subtropical in composition, including palms (*Sabal* sp.), *Laurus*, *Cinnamomum*, even *Nipa*, the Sarmatian flora of the Sea of Azov has given the following plant association: *Muscites* sp., *Pteris* sp., *Salvinia* sp., *Taxus baccata*, *Pinus palaeostrobus*, *Taxodium distichum miocaenicum*, *Sequoia Langsdorfii*, *Typha latissima*, *Potamogeton* sp., *Phragmites oeningensis*, *Arundo Goepfertii*, *Poacites cf. angustus*, *P. cf. caespitosus*, *P. cf. laevis*, *Cyperites cf. Deucalionis*, *C. cf. paucineris*, *Amsoneuron*, *Noeggerathiae*, *Smilax grandifolia*, *Juglans acuminata*, *Hicoria bilinica*, *Carpinus grandis*, *Corylus Macquarrii*, *Alnus Kefersteinii*, *Betula macrophylla*, *Castanea Kubinyi*, *Fagus Deucalionis*, *Quercus pseudocastanea*, *Populus balsamoides*, *Myrica* sp., *Ulmus* sp., *Celtis trachytica*, *Zelcova Ungerii*, *Ceratophyllum Sniatkovii*, *Ranunculus* sp., *Laurus Guiscardii* (aff. *L. cana-*

riensis), *Platanus aceroides*, *Parottia pristina*, *Ficus cf. wetteravica* (?), *Sassafras ferretianum*, *Crataegus praemonogyna*, *Eucommia ulmoides*, *Prunus Luculli*, *Cercis siliquastrum*, *Ailanthus Confucii*, *Rhus quercifolia*, *Sapindus Hazslinskyi*, *Acer laetum*, *A. sanctae crucis*, *A. subcampestre*, *Vitis praevinifera*, *Firmiana tridens*, *Cornus sanguinea*, etc. The flora has essentially a Japano-Chinese aspect with some eastern American alliance; and if certain evergreen plants are present, they do not play any important role, and belong to the same range of floras.

Notwithstanding such comparatively complete knowledge of both floras, European and Transuralian, we have no means of making a proper comparison between them.

Most fortunately, through the efforts of the geologists Messrs Vakhrushev, Gerasimov, Vodianikov and Miss Tiazheva, there has at last been found, on the western side of the Urals, a flora which makes a strong link between both regions, the Asiatic or Turgayan proper, and the European, or Poltavian, the latter being later invaded by the Turgayan. This link I find in the locality, discovered in Bashkiristan (formerly the province of Ufa) not far from the town Sterlitamak, at the sites Shkatly, Romodanovka and Kinziabayeva. The flora found there contains the following species: *Ginkgo adiantoides*, *Taxodium distichum miocaenicum*, *Sequoia Langsdorfii*, *Glyptostrobus europaeus*, *Phragmites oeningsensis*, *Fagus Antipovii*, *Castanea Kubinyi*, *Quercus Nimrodi*, *Q. nerifolia* (abundant¹), *Liquidambar europaeum*, *Platanus aceroides*, *Alnus Kefersteinii*, *Betula macrophylla*, *Comptonia dryandroides*, and several others.

It is easy to see that the composition of the Sterlitamak flora corresponds, even in detail, with that of the Turgayan flora of the *Liquidambar* type. If a few small changes or additions should be made later its general type does not differ in any way from the usual type of the Aquitanian flora, which is found across the whole of Asia with small variations consequent on conditions of time or space. For instance *Comptonia*, which on the Island of Sakhalin is of the type *C. acutiloba*, here changes to *C. dryandroides* with somewhat narrower lobes.

However, even the tracing of the Turgayan Aquitanian flora into Europe does not decide the problem of its age without further comparison of the Sterlitamak flora itself, as well as of the Asiatic Turgayan in general. But proofs are at hand if we use the Krynka flora for comparison. This is of well-determined age, having typical Sarmatian fauna in the beds closely associated, and contains numerous

plant species, listed above. By comparing the two floras, those of Krynka and Sterlitamak, two places on the eastern boundary of Europe, but the former about 7° farther south than the latter, we may reach valuable conclusions. Both floras are temperate and composed predominantly of deciduous elements; also to a great extent the genera and to some extent the species coincide. Nevertheless, on a more precise analysis of the two floras we notice that in the Sterlitamak flora certain evergreen plants such as *Quercus neriifolia* play rather an important role, while this plant is entirely absent from the Sarmatian flora of the Sea of Azov. Further, the Sterlitamak flora contains some definitely archaic elements such as *Fagus Antipovii*, while in the Sarmatian only *F. Deucalionis* is represented. Even were the resemblance of the two floras, the Krynka and the Sterlitamak, exact, the conclusion would be inevitable that the more northern of the two, that is the flora of Sterlitamak, is the older. But the fact already stated that certain Poltavian elements, lacking in the Sarmatian flora of Krynka, played an important role on the western slope of the Urals, shows that the Sterlitamak flora is the earlier of the two, and must belong to the earlier stage which I regard as the Lower Miocene or Aquitanian (Upper Oligocene), assuming that the whole complex of the Turgayan flora remained unchanged, but was enriched by certain evergreen Poltavian elements which remained behind when the rest of the Poltavian flora was driven south and south-west. From another point of view this statement as to the age of the flora of Sterlitamak confirms the theory that the Asiatic Turgayan flora, for instance that of the Aralo-Caspian region, is really older than Miocene, and that G. Saporta⁽³⁾ and S. Gardner⁽⁴⁾ were right in their opposition to O. Heer, who considered the Miocene flora as a monotonous conservative association lacking any dynamics and distinctions in the whole of its immense area.

It is most important that all the Tertiary floras of eastern Europe acquire quite a definite position when they are compared with the Uralian flora now under consideration, due account being taken of its age and composition. All the Sarmatian (Upper Miocene) floras, those of the lower Don, Krynka, Orickhov on the Konka river⁽⁵⁾, northern Bessarabia and Podolia, which are associated with a well-determined marine fauna, look considerably younger than the Sterlitamak flora and have a more modern aspect, being closely allied to living floras, though these floras inhabit distant regions—China, Japan and the eastern U.S.A. Still younger and more modern

in their composition are the Maeotic or Early Pliocene floras of Odessa, Bessarabia, etc., though the floras of the same age known from the same strata, but from regions more to the south—Caucasus(6), Bulgaria—display a more southern aspect, being more nearly related to the older floras of regions to the north.

In contradistinction to this all the floras of the European part of the Soviet Union belonging to the Oligocene or Eocene, broadly speaking the Poltavian flora, appear entirely alien both as regards the Sarmatian as well as the Uralian flora we have been considering, signifying the advent of the Turgayan flora into Europe across the Uralian barrage after the desiccation of the Tertiary sea along its eastern slope. As we know, all the Poltavian floras appear more "exotic"—although really they are much more "endemic" or aboriginal here—being in general evergreen and having a conspicuous Indo-Malayan aspect, later disappearing during gradual substitution by the Turgayan elements which are the true "exotics".

I do not mean to say that all broad-leaved temperate elements have penetrated into Europe *via* the Urals, which nevertheless was a very important way for eastern Europe. It is most probable that western Europe in great extent has received its temperate elements directly from the Arctic—Iceland, Spitsbergen, *via* Scandinavia—whose more temperate vegetation was established to the north of semi-tropical western Europe from early Tertiary times. In my opinion this fact results from the Arctic and northern and eastern Asia being integral parts of one phytogeographical zone. I can scarcely separate this process from the movement of the geographical latitudes to the south-west: or more correctly from the swinging of western halves around a nearly immovable point situated somewhere in south-eastern Asia, in which case the Indo-Malayan region did not change in any considerable degree either its geographical condition, climate, or type of flora. It is quite natural that the swinging of the geographical latitude was most rapid in western Europe, which fact markedly coincides with the rapid change in the composition of the west European flora during the Tertiary, whilst in Asia this process was proceeding much more slowly and insignificantly. The flora of north-eastern Asia preserved its salient features from the Uppermost Cretaceous until the conditions of the Glacial Period drove the aboriginal flora to the south, or entirely exterminated it when safe exodus was barred by insurmountable obstacles.

It is for this reason that the Tertiary flora of eastern and central Asia seems to be so monotonous. For the same reason the temperate

flora of China, Japan, and to some extent of the Maritime Province of the Soviet Union, is really an aboriginal Tertiary flora, though somewhat depauperated; while the temperate flora of Europe is only a secondary descendant of the Tertiary ancestors. Whilst we can describe the first as a real Tertiary flora as regards its generic and specific components, the second is only a Quaternary flora having all its species of later origin, although possibly most of them were ready at the very beginning of the Quaternary period.

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REVIEWS

Diseases of the Banana and of the Manila Hemp Plant. By C. W. WARDLAW, Ph.D., D.Sc. 8.9×6.5 in. Pp. xii+615, with a coloured frontispiece and 292 text-figures. London: Macmillan and Co. 1935. Price 30s.

Much attention has been paid in recent years to the study of the diseases of the major tropical crop plants. Evidence of this activity is afforded by the publication of several monographic accounts of the diseases of particular crops which are cultivated on a large scale. The latest addition to this series is Dr Wardlaw's book on the diseases of the banana and of the allied fibre plant, Manila hemp. Of all tropical crops perhaps the banana plant has been the most heavily afflicted by really grave diseases. In the West Indies and Central America cultivation of the Gros Michel variety, the most valuable one commercially, is seriously threatened by Panama or wilt disease, which is caused by the soil-inhabiting fungus *Fusarium cubense*. In certain parts of Queensland and New South Wales a virus disease, known as bunchy top, has rendered derelict large banana areas. In addition, diseases of the fruit in transit across the seas sometimes occasion great losses.

No one is better equipped than Dr Wardlaw to write a book on banana diseases, for he has spent several years in the West Indies investigating these troubles and he has travelled widely in other countries where the crop is grown extensively. In this book the diseases of the banana both in the field and in storage are treated exhaustively, and there is a complete citation of the numerous papers which have been written on the subject. With regard to Manila hemp, the diseases of the plant in the field, including a serious virus disease allied to bunchy top, and the deterioration of the fibres in storage by fungus attack are described.

To plant pathologists perhaps the most interesting part of the book will be the account of the author's investigations and personal outlook on the devastating wilt disease of bananas. Dr Wardlaw takes a less pessimistic view of the spread of this disease than do certain other mycologists who have investigated it, for he holds that if the plant is growing in a well-aerated and uniformly moist soil it cannot be successfully attacked by the fungus owing to the barriers made by the host under these conditions to attempted invasion. Whether the future upholds this opinion or not, the author has rendered a great service in emphasising that the disease is worse on some types of soils than on others. In view of the great difficulty of direct mycological control of this wilt efforts are being made in Trinidad and Jamaica to breed a variety of banana which will possess the commercial qualities of Gros Michel and which will also be resistant to *Fusarium cubense*; the book contains a useful summary of the results so far achieved.

Although the book is a most valuable treatise on the maladies which afflict this important tropical fruit, it is unnecessarily lengthy. The author goes into such minute details of his own and of others' investigations that it is sometimes difficult "to see the wood for the trees". Long quotations are given from published papers. These are superfluous in a book of this kind, for those specially interested can always consult the original papers. It would have been better if the author had confined himself to essential facts and to a critical review of current opinion on the pathology of the banana and Manila hemp: as it is, the author's viewpoint is partially obscured by a maze of detail.

The book is profusely illustrated, but some of the photographs are insufficiently clear. The author's style, although prolix, is generally lucid, but there

are a few awkward expressions, such as "the disease manifests itself by a very often enormous distension of the base of the stem" (p. 160). In referring to *Thielaviopsis paradoxa* it is incorrect to say that this is "the cause of pineapple disease" (p. 436), for several different fungi cause disease in pineapples. The book is almost entirely free from misprints.

F. T. BROOKS.

Botany. Principles and Problems. Third ed. By E. W. SINNOTT. 9×6 in. Pp. xix+525, with frontispiece and 310 figures. New York and London: McGraw Hill Book Co., Inc. 1935. 21s. net.

It has evidently been the intention of the author of this book that it shall be stimulating and "different". Remarking that a "just balance between caution and enthusiasm is hard to strike" he is yet prepared to give ¹⁴st some hostages to fortune. The result can hardly be accused of dullness. He has no patience with "quaint individuals who wander absent-mindedly in woods... and rejoice in the use of long and unpronounceable Latin words", though in passing he does invent "Tracheophyta".

For a year's course the text sets wide limits. The history of botany, the nature of soils, morphogenesis and plant associations are some of the subjects introduced and not always found in elementary text-books. The method is essentially wide rather than thorough, and the book would be suitable for those whose knowledge of botany is to be rounded off at an elementary stage as well as for those who are to continue on a "concentric" system.

Practical work is not considered, but each chapter ends with questions for "thought and discussion". Some of these really should stimulate both, and, in the words of the author, help the student "to be his own Socrates". Others appear to me unanswerable.

The later chapters, dealing with groups and types of the plant kingdom, occupy nearly a third of the book. Besides descriptions of selected plants they deal at some length with origins and evolutionary tendencies within groups such as the angiosperms. This is probably a heavier weighting than the subjects would get in any elementary course in this country, where the present tendency is setting so strongly in favour of experimentalism. Similarly, the account of mitosis is of a kind that many would think dated.

Up and down the book there are a number of statements rather surprising in a third edition; e.g. within a few pages, "It is probably the protoplasm itself... which is oxidised"; "Most of the energy liberated in respiration eventually manifests itself as heat" (surely a wrong emphasis); "The resulting alcohol may be absorbed by another organism or may be burned" (not a satisfactory synonym for biologically oxidised). The general treatment of xerophytes, described as "drought-loving" plants, is decidedly disappointing in view of the recent interest and advances in studies of such types.

Great care has evidently been lavished on the illustrations, which consist of over three hundred half-tones and line drawings. Sixty have been specially prepared for this new edition. All are excellent with the exception of the flower drawings, and these are horrible.

Though it is improbable that any English botany teacher would wish to build his course round this book, it might well be put into the hands of students, especially students requiring a strong botanical stimulant, to provoke a flow of "thought and discussion". It would surely bring this about.

W. O. JAMES.

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ACTIVATION OF CAMBIAL GROWTH BY PURE HORMONES

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(With 5 figures in the text)

IT was shown by Jost (1891, 1893) that cambial growth depends on some influence coming from the leaves, and especially the growing leaves, which travels only in a morphologically downward (or obliquely downward) direction, and is distinct from the general supply of nutriment. The writer showed recently (1933) that this influence can pass a protoplasmic discontinuity and is almost certainly a soluble substance of the nature of a hormone.

Recently also Laibach (1933) caused decapitated epicotyls of *Vicia Faba* and various petioles to increase in thickness by placing on them the pollinia of orchids, which he has shown to exude large amounts of an auxin, a hormone which promotes the elongation of stems. Continuing these experiments, Laibach, Mai and Müller (1934) have briefly reported that in stems of *Coleus* and *Tradescantia* swellings can be caused by applying orchid pollinia or an ether extract of pollinia or of urine, taken up in lanoline, and further that these swellings involve formation of callus and numerous cell divisions. Similar results with other species are mentioned by Mai (1934) and Müller (1935). In these papers the authors leave open the question whether the substance which provoked the cell divisions was the auxin contained in the pollinia or extracts. Subsequently Laibach (1935), in a very recent paper, has reported that he has caused very vigorous formation of callus and roots in stems of *Vicia Faba* and *Coleus* by applying to them, mixed with lanoline, solutions of synthetic hetero-auxin (that is, β -indolyl-acetic acid) of very high concentrations, up to 1 part in 500. (The fact that hetero-auxin promotes root formation had been shown already by Thimann and

Koepfli (1935).) Some further experiments with hetero-auxin are reported by Laibach and Fischnich (1935). But in none of these papers have Laibach or his collaborators stated whether cambial growth of a normal kind and in the normal position, as distinct from irregular cell divisions and formation of callus, was activated by the substances applied.¹

For the purpose, therefore, of identifying the cambial hormone, it seemed to the writer desirable as a first step to determine whether a hormone capable of activating cambial growth is contained in urine, since urine contains abundant auxin and other hormones also. Accordingly the ether-soluble component of urine was extracted for this purpose by the method of Kögl and collaborators (1933). I am much indebted to Dr Weissberger, of the Dyson Perrins Laboratory, Oxford, for kindly constructing and showing me the use of the necessary apparatus.

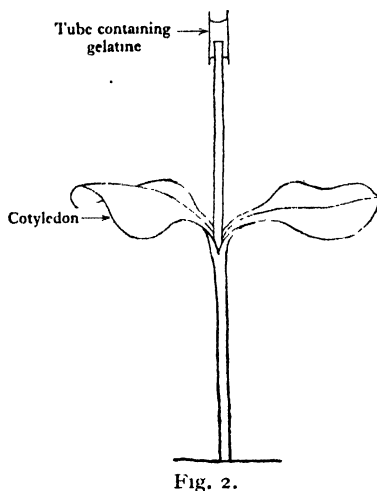
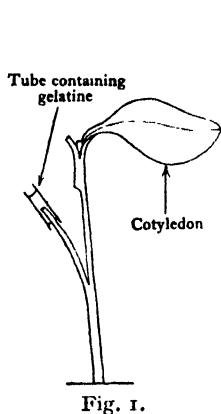
The extract was tested on young sunflower seedlings. The simplest way of testing, which was used in a later experiment, is to decapitate a seedling near the top of the epicotyl, when this has elongated, and to apply a solution of the substance to the upper cut surface, as is shown in Fig. 2. But in the present experiment (Exp. 1), since the time of year was November and it was doubtful whether there was enough light for photosynthesis, it seemed better to apply the substance to the hypocotyls of very young seedlings, well before the reserves of the cotyledons were exhausted. The method, which is shown in Fig. 1, was the following.

When the first pair of leaves were about 20 mm. long, the seedlings were decapitated above the cotyledons, and the hypocotyls were split for about 25 mm. in the median plane at right angles to the plane of the cotyledons. One of the split halves was cut through at the top so that it remained as an upward-pointing strip (see Fig. 1), and the cotyledon that was vertically above this strip was removed. The top of the split was about 5 or 10 mm. below the cotyledons. The extract was now applied to the upper ends of the upward-pointing strips at a concentration of 2 parts in 10^4 in the following way. It was dissolved in a 25 per cent. solution of gelatine, which contained also thymol at a concentration of 1 in 10^5 as antiseptic, and was kept just warm enough to remain liquid. The solution was introduced with a glass rod into short pieces of glass tubing open at both ends and

¹ The writer has not been able to see a paper by Laibach in *Wiss. Woch. Frankfurt a. M.* vol. 11, p. 67, 1935. Recent papers by Czaja (1935*a, b, c*) will be commented on at the end of the present paper.

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containing about 0.15 c.c., which were then slipped over the ends of the upward-pointing strips, the seedlings being meanwhile bent downwards if necessary. After a short time the gelatine solidified, and the tubes were then sealed with wool-fat below and vaseline above: the exposed cut surfaces were also vaselined. After every 2 or 3 days, the tubes were removed, the upper ends of the strips were wiped clean and fresh solution was applied. As controls other similar seedlings were operated on and treated similarly, except that the solution of gelatine and thymol which was applied contained no extract of urine.



After 19 days it was found that in the "experimental" plants—that is, those which had received the extract of urine—the zones which had been covered by the gelatine (which were about 8 mm. long) were clearly swollen, and in transverse sections through these parts it could be seen that vigorous cambial growth had taken place: for in the bundles there was a cambial zone about 6 cells deep, and between the bundles a rather irregular cambial zone from 2 to 8 cells deep. Also in two of the plants secondary xylem had been formed, in regular radial cell rows, and in one of the plants the rows were more than 12 cells deep. In the inner cortex also there had been numerous cell divisions, and at various points lateral roots had been formed and had grown out through the cortex to the surface. In the controls, sections through the zones covered by the gelatine showed no cambial growth, nor root formation, nor secondary cell divisions of any kind.

At a level 2 or 3 mm. below the parts covered by the gelatine, the cambial growth in the hypocotyl strips of the experimental plants had been much less vigorous, but a cambium had been formed across most of the main gaps between the bundles in the normal position, and in some of these gaps the cambial zone was 3 or 4 cells deep. At this level there were no divisions in pith or cortex. The controls had formed no divisions at this level either.¹

The fact that the effect of the extract extended only a little way below the parts to which it was applied may have been due to lack of light. In later experiments with better illumination a much better transport of hormone was obtained.

Since the ether extract of urine had been found to stimulate cambial growth, the next step needed was to determine whether or not the effective substance was identical with auxin-*a*, which is contained in urine. For this purpose, Prof. Kögl very kindly sent to the writer 0.1 mg. of pure auxin-*a*, and this was at once tested for its effect on cambial growth at a concentration of 2 parts in 10⁶. It was applied, in gelatine with thymol, to the upper ends of hypocotyl strips of two young sunflower seedlings (Exp. 2), by the method of the previous experiment, and also (Exp. 3), the time of year being now March, to the upper ends of two slightly older sunflower seedlings, which were decapitated at a level 10 mm. below the top of the epicotyl when their cotyledons were fully grown and their first leaves were 50 or 60 mm. long (see Fig. 2). The gelatine containing the hormone was renewed every 3 days. It was dissolved in tap water, of pH 7.9, and it was not found necessary to acidify the solution. To each pair of seedlings there were two controls similarly operated upon and of the same age, but these did not have gelatine and thymol applied to them, since the previous experiment had shown that gelatine and thymol alone have no effect.

After 16 days it was found that in the experimental plants which had been operated upon in the hypocotyl (Exp. 2) there had been very vigorous cambial growth in the hypocotyl strips, both in the parts covered by the gelatine and hormone (which were 4 or 5 mm. long) and also at a level 6 or 8 mm. below these parts. For at both these levels there was a cambial zone from 4 to 8 cells deep, both in and between the bundles, and secondary xylem, containing many

¹ The results of this experiment were briefly reported previously (Snow and Le Fanu, 1935), and it is clear that they cannot rightly be described as a formation of callus from a cambium, as Laibach (1935) has understood them. For the products of the cambium, where they had differentiated, were not callus, but xylem on the inner side and apparently phloem on the outer side.

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vessels, had also been formed and was from 6 to 8 cells deep in the parts covered by the gelatine and from 3 to 6 cells deep at the level 6 mm. below. One of these seedlings had formed five roots in the part covered by the gelatine. The hypocotyl strips of the two corresponding controls had formed no cambium, neither in the bundles nor between them, except that in a part of one bundle one of them had formed a small patch of cambium about 2 cells deep. The cambial growth in the "experimental" seedlings had even been very much stronger than in two intact seedlings of the same age. In the "experimental" seedlings there had also been some cell divisions in pith and cortex in the parts covered by the gelatine and auxin, but scarcely any at the level 6 mm. below.

In the two seedlings which had had the gelatine and auxin-*a* applied to the upper ends of their epicotyls (Exp. 3), the effect of the hormone could be traced for at least 20 mm. down the epicotyl, and in one of them for at least 30 mm., as is shown by Table I which gives the depth of the cambial zone (measured by the number of cambial cell walls) in the main gaps between the bundles, at levels 10, 25 and 35 mm. below the upper cut surface. The uppermost 4 or 5 mm. were covered by the gelatine and hormone. Corresponding figures are given for the epicotyl stumps of the two controls at various levels.

TABLE I. *Depth of cambial zones in main gaps between bundles of epicotyls at various levels, measured by number of cambial cell walls.*

	10 mm. below top	25 mm. below top	35 mm. below top
Experimental plant No. 1	4	4 in most gaps, 2 or 3 in some gaps	2-4
„ No. 2	2 in most gaps, 3 in others, 1 in some gaps: at a few points incomplete	2 in most gaps, 1 in others, 0 in some gaps	2 in most gaps, 1 in a few gaps, none with 0
Control No. 1	5 mm. below top, 0	15-20 mm. be- low top, 0	(Epicotyl only 30 mm. long)
„ No. 2	0 in most gaps, 1 in a few gaps	0 in most gaps, 1 in a few gaps	1 in most gaps, 2 in a few gaps, 0 or incom- plete in se- veral gaps

Parts of transverse sections of the first of the experimental plants, at two different levels, and of one of the controls are illustrated in Figs. 3, 4 and 5. For these drawings, which were drawn under

projection apparatus, I am much indebted to my wife. Each figure shows parts of two bundles together with the region between them.

From the table it can be seen that in the epicotyl stump of one of the controls there had been no cambial divisions in the gaps between

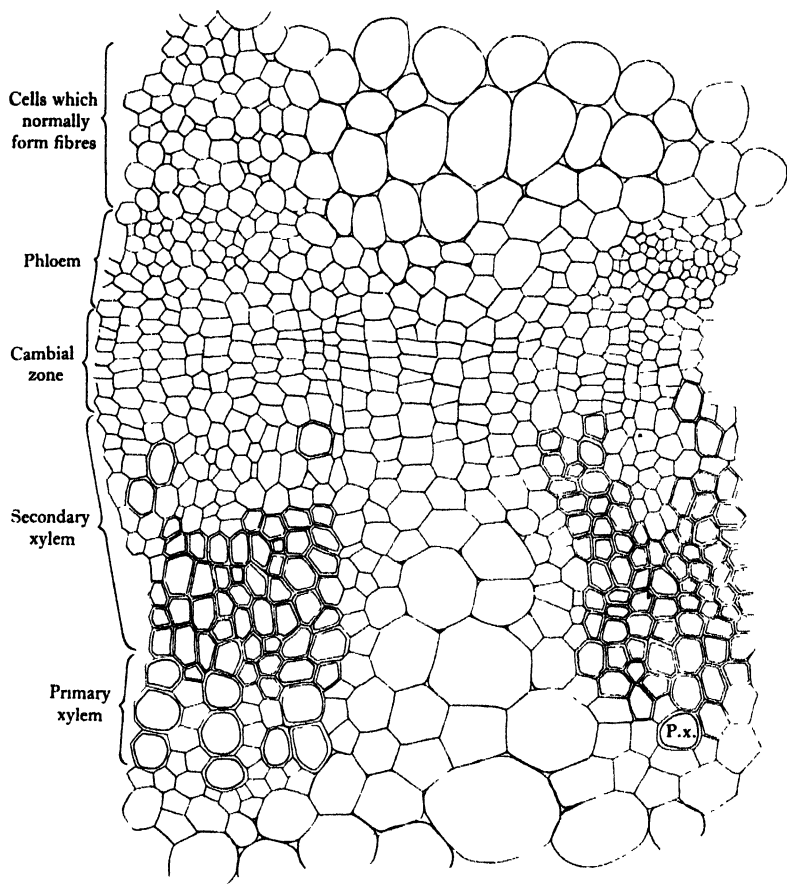


Fig. 3. Exp. 3. Experimental plant no. 1. Section at 2 mm below top, in zone covered by gelatins and auxin- α . $\times 202$.

the bundles, while in the other control there had been no divisions in most of the gaps, but one division in a few of them: this had probably taken place before the decapitation. Within the bundles there had been no cambial divisions, except near the base of the epicotyl of one of the plants, where there had been 1 or 2 divisions in one bundle. On the other hand in the two experimental plants there had been considerable cambial growth between the bundles, even at levels 25 and

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35 mm. below the top. The depth of the cambial zone in the bundles was about the same, or slightly greater. The slight break and irregularity in the cambial zone between the bundles, which is shown in Fig. 4, is of a kind which has been observed in intact seedlings also.

At the extreme upper ends of the epicotyls, where they had been covered with the gelatine and auxin-*a*, there were large swellings, in which there had been very vigorous cambial growth and formation

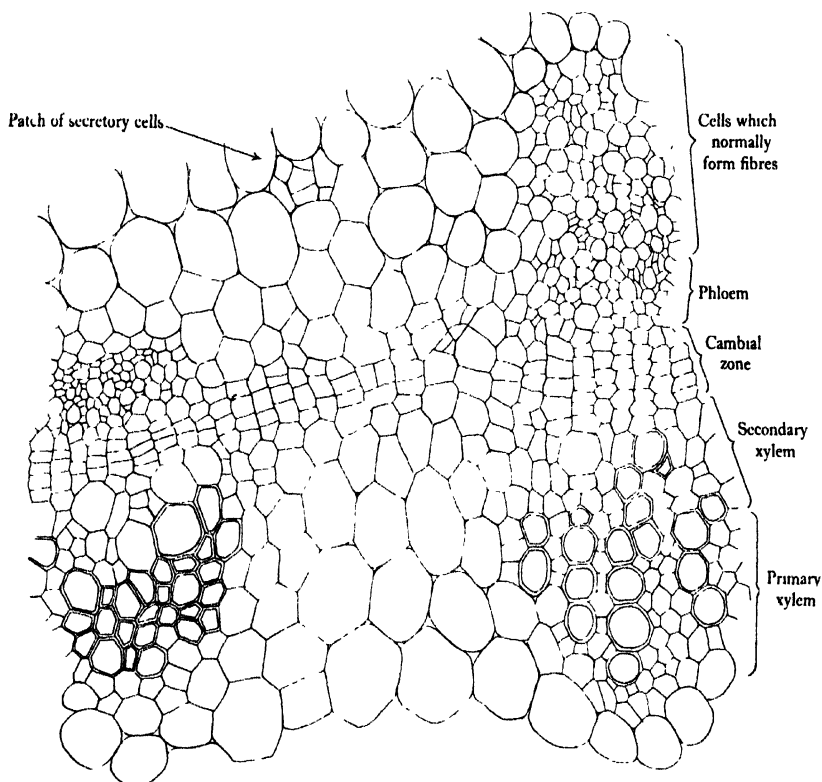


Fig. 4. Exp. 3. Experimental plant no 1. Section at 10 mm. below top, and 5 mm. below zone covered by gelatins and auxin-*a*. $\times 202$.

from it of secondary xylem in the bundles as can be seen from Fig. 3. The secondary xylem, as can be seen from Fig. 3, contained many vessels and was about 8 cells deep in the plant illustrated, and from 2 to 4 cells deep in the other experimental plant. At 5 mm. below the zone covered by the gelatine, also, one of the experimental plants showed several layers of secondary xylem containing vessels, as can be seen from Fig. 4.

The secondary xylem at both levels was of normal kind, so far as could be judged, except that lignification had proceeded in it more slowly than in the secondary xylem of intact sunflower seedlings. Also the extensive groups of cells in the outer part of the phloem, which in intact seedlings thicken their walls and are converted into conspicuous fibres, had remained thin-walled in the experiments just as in the decapitated controls. From this it follows that the thicken-

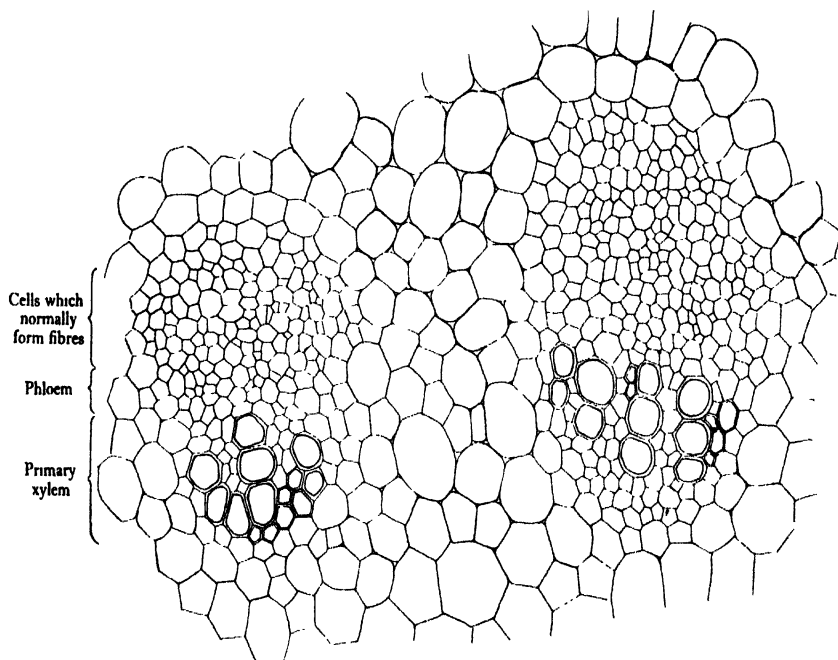


Fig. 5, Exp. 3. Decapitated control plant no. 1. Section at 15 mm. below top. $\times 202$.

ing of the walls of phloem fibres depends on some influence coming down from above which is different from the cambial hormone.

It should be noted that in these two experimental plants there had nowhere been any cell-divisions in pith or cortex, not even in the parts covered by the gelatine and hormone: the divisions were entirely limited to the cambial zone. From these results it is clear that auxin-*a* at a concentration of $2 \text{ in } 10^6$ activated strong cambial growth, and further that its effect travelled in two of the plants for at least 20 and 30 mm. downwards.

It is known that various fungi, including *Rhizopus* (Nielsen, 1930) and yeast (Kögl, 1935), form a substance which acts on plants in the

same way as auxin-*a* in all respects which have so far been investigated. This substance has been isolated from yeast by Kögl and Kostermans (see Kögl, 1935) and has been found to be β -indolyl-acetic acid. It is called by Kögl "hetero-auxin", and its efficiency in promoting the elongation of coleoptiles is about half that of an equal amount by weight of auxin-*a*.

It seemed of interest to determine whether hetero-auxin would also promote cambial growth, and Dr Weissberger, of the Dyson-Perrins Laboratory, Oxford, very kindly synthesised a generous supply of crystals of it for the writer, who is much indebted to him for so doing. It was tested at a concentration of only 1 part in 10^6 on three young sunflower seedlings (Exp. 4). The method used was that shown in Fig. 1, the time of year being February, and the gelatine containing the hormone was renewed every 3 or 4 days.

After 21 days it was found that in the strips of hypocotyl of the "experimental" seedlings there had been fairly vigorous cambial growth, and that this had been about equally vigorous in the part covered by the gelatine and hormone (which was 8 or 9 mm. long) and at a level 6 mm. below the base of this part. Thus at the lower level one plant showed in the main gaps between the bundles a zone of 4 or 5 cambial cell walls in depth, another showed a zone of from 1 to 3 cambial cell walls in depth, and the third showed in one gap a zone of 4 cambial walls and in another gap a zone with 1 cambial wall. The cambial zone *in* the bundles was about equally deep. In two controls of the same age, similarly operated upon, there had been no trace of cambial growth, neither in the bundles nor between them. Thus hetero-auxin, at a concentration of 1 in 10^6 , also activates cambial growth.

In these "experimental" plants there had been no divisions in the cortex, or scarcely any, even in the part covered by the gelatine and hormone. But there had been some divisions in the pith in this part. The results obtained with auxin-*a* and hetero-auxin were briefly reported in *Nature* (Snow, 1935).

DISCUSSION

The results show clearly that ether extract of urine, auxin-*a* at concentration 2 in 10^6 , and hetero-auxin at concentration 1 in 10^6 activated cambial growth in stems of sunflower seedlings, and that the effect of these hormones travelled down for some distance below the parts to which they were applied—in one seedling for 30 mm. at least. The cambium formed was always in the normal position, and

in the parts covered by the gelatine and hormone it grew very vigorously and often produced on the inner side many layers of xylem parenchyma, and sometimes vessels also, in 3 weeks or less. In these parts also (though not at the lower levels) there were often root formation and cell divisions in the inner cortex and pith. Thus the same hormones, auxin-*a* and hetero-auxin, which promote cell extension in stems, also activate cambial growth, besides activating the formation of lateral roots (Thimann and Went, 1934; Thimann and Koepfli, 1935), and producing various other effects.

The results support the conclusion reached previously (Snow, 1933) that the influence coming down from the leaves which activates cambial growth is a hormone. This hormone may actually be auxin-*a*, since its properties are the same, so far as they have been investigated, and Kögl (1935, p. 22) has shown that the hormone formed by the tips of coleoptiles is probably auxin-*a*. But whether or not the cambial hormone is auxin-*a*, it may reasonably be concluded that it is the same as the "growth hormone" formed by shoots, which promotes cell extension. For firstly cambial growth and cell extension, as has now been shown, can be activated by the same hormones; and secondly the cambial hormone and the growth hormone are both formed by leaves and mainly by growing leaves and buds, as has been shown by the numerous observations of Jost (1891, 1893) for the former hormone, and by those of Thimann and Skoog (1934, p. 319) for the latter.¹

But in order to check this conclusion further, it is worth while to compare the amounts of hormone applied in the experiments with the amounts formed naturally. Thimann and Skoog have found (1934, pp. 319-20) that in *Vicia Faba* the terminal buds and the growing leaves together secrete on an average about 40 "plant units" of growth hormone per hour. Now one "Avena-unit" of Kögl and his collaborators equals about 2 "plant units" of Thimann and his collaborators and one "Avena-unit" contains about $\frac{1}{5 \times 10^7}$ mg. of auxin-*a* (Kögl and others, 1933). Therefore the amount of growth hormone secreted per hour into a shoot of *Vicia Faba* is roughly equivalent to $\frac{40}{2} \times \frac{1}{5 \times 10^7} = 0.4 \times 10^6$ mg. of auxin-*a*.

¹ In *Acalypha* the old leaves apparently continue to produce comparatively large amounts of hormone (see Boullenne and Went, F. W., 1933, pp. 93 *seq.*). The comparative efficiencies of young and old leaves perhaps vary greatly in different species.

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Now in the experiment with hetero-auxin here reported, about 0.15 c.c. of a solution of hetero-auxin of 1 part in 10^6 was supplied to each strip of sunflower hypocotyl every 3 or 4 days. If therefore it is supposed (as a maximum estimate) that all the hormone passed into the hypocotyl, the amount so passing in was 0.15×10^{-6} gm. every 3 days, $= 2 \times 10^{-6}$ mg. of hetero-auxin per hour: and this is about equivalent to 1×10^{-6} mg. of auxin-*a* per hour.

Thus the amount of hetero-auxin supplied per hour to the strips of sunflower hypocotyl was at most equivalent to about 2.5 times the amount of growth hormone secreted per hour by the terminal bud and growing leaves of a *Vicia Faba* seedling, and was probably considerably less, since there was probably considerable wastage.

In the experiments with auxin-*a*, the amount supplied was about twice as much by weight as in the experiment with hetero-auxin, and this is equivalent to an amount about four times as great.

These calculations show that the amounts of hormone which activated cambial growth in the experiments are not very different from the amounts secreted naturally: the cambial growth was more than the normal.

It is a little surprising to find that auxin-*a* and hetero-auxin promote not only cell extension, but cell division also. But it may be supposed that these hormones exert some primary effect from which there may follow secondarily either cell extension or cell division or both, according to the nature and condition of the tissue on which they are acting. Naturally the continued growth of a cambium must involve a slow extension of cell-wall material in the radial direction, which keeps pace with the cell divisions.

Another problem which was pointed out previously (Snow, 1932, p. 351) concerns the directions of transport of hormone. For in stems, as was shown by Jost (1891, 1893), the cambial stimulus travels only in the morphologically downward (or obliquely downward) direction, and the same rule appears to hold for the fully elongated parts of roots, as the writer has found. Also the growth hormone, as revealed by its effect on elongation, has so far been found to travel only (or mainly) downwards in stems and coleoptiles, but in roots it travels *upwards* from the tip to the elongating region. Indeed Cholodny (1934) claims to show that in this young zone of the root it travels *only* upwards. If then the cambial hormone is the same as the growth hormone, how are these facts to be explained? They would be explained if it were found that the growth hormone is transported

downwards in the stem and in the part of the root above its elongating zone, but upwards in the part below that zone.¹

It remains for the present unexplained how it is that in intact plants a cambium is formed only in a certain position in the cross-section of stem, where also it was formed in the present experiments. Perhaps the parenchyma cells between the bundles are for some reason more susceptible to the action of the hormone than the cells of pith and cortex. In the present experiments numerous cell divisions were indeed found in pith and cortex in some (not all) of the plants, though only in the zones covered by the gelatine and hormone and not lower down. But these divisions were irregularly orientated and quite different from the cambial divisions which took place in the normal position.

In a paper received after the present paper was written, Czaja (1935*c*) reports, in agreement with Laibach and collaborators (1934, 1935), that sources of growth hormone, when put on the tops of stems, cause growth in thickness and cell divisions; but he offers a different explanation. He supposes that the growth hormone coming down from above meets another stream of growth hormone which he believes to come up from the cotyledons below, and further that the two streams meeting cause a disturbance of polarity, which in turn for some reason causes cell divisions or even local cambial growth. Although some of the experimental results reported by Czaja in this and other recent papers (1935*a, b, c*) are of great interest, this theory does not seem to the writer to be supported by any adequate evidence. The fact, which he reports, that his epicotyl stumps did not swell or show cell divisions if the cotyledons were removed could be interpreted differently. Also the theory meets with the obvious difficulty that most workers have found that the growth hormone cannot be transported in the morphologically upward direction in stems, or only to a slight extent. But further comment may be reserved until the full account is published.

In any case, whatever may have been the process by which the hormones activated cambial growth in the experiments here reported, whether direct or indirect, there is no reason to suppose that it was different from the process by which the hormone descending from the leaves normally activates cambial growth. For the hormones sup-

¹ Czaja (1935*b*) has already suggested that these are the normal directions of movement of the growth hormone in these parts, though he also believes (1935*a, b, c*) that both stem and root are capable of transporting the hormone in both directions. His theory will be commented on later.

plied in the experiments were travelling down at concentrations not very different from the normal; and the cambial growth activated by them appeared to be of just the same kind as the normal (except that the cells formed by the cambial growth differentiated less rapidly) and it was in the normal position. In the experiments of Laibach and others of his school, already mentioned, the concentrations of growth hormone applied were often or always very much higher than in the present experiments; and it is probably for this reason that the anatomical changes reported by these workers have so far all (except the formation of lateral roots) been of a rather abnormal kind, and have apparently obscured any cambial growth of a normal kind which may have taken place.

SUMMARY

1. Cambial growth was activated in decapitated stems or hypocotyls of young sunflower seedlings by applying to their upper ends solutions in gelatine of pure synthetic hetero-auxin and of pure auxin-*a*, at concentrations of 1 in 10^6 and of 2 in 10^6 respectively. The cambium formed was in the normal position and of the normal kind. In one experiment the effect of the hormone (auxin-*a*) extended for at least 30 mm. below the region to which it was applied.

2. The results are discussed, and it is concluded that normally cambial growth is activated by the same growth-hormone which, formed by the young leaves, promotes extension of cells in stems. This hormone may actually be auxin-*a*.

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ON THE STRUCTURE, DEVELOPMENT AND
DISTRIBUTION OF THE ENDODERMIS
AND ITS ASSOCIATED DUCTS IN
SENECIO VULGARIS

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(With 13 figures in the text)

INTRODUCTION

ALTHOUGH anatomical and experimental studies have been made on the roots and stems of a large number of plants from the standpoint of endodermal characters, few detailed studies of the distribution of the endodermis and its associated structures in any one plant as a whole seem to have been studied. With this in mind, the present work on *Senecio vulgaris* was undertaken.

The fact that this plant is an ephemeral made it possible to investigate the development and distribution of the endodermis in plants grown to maturity under different seasonal conditions. The most striking result of this investigation was that a difference in extent of the endodermis was found in corresponding parts of plants which had developed at different seasons.

The plants used were obtained from various localities in the East Midlands. Under natural conditions widespread crops of seedlings occur in this area about the end of April. The normal period of growth of these plants from germination to seed production is from 9 to 11 weeks. The next widespread crops of seedlings appear in September. These latter plants live through the winter and flower over a more prolonged period than the summer plants. In addition, mature flowering plants are found which are probably survivors of the summer crop. These plants are characterised by strong lateral shoots and are of a robust habit.

While it is not possible to make an absolutely clear-cut distinction between the characteristics of the spring and autumn plants, the

former are predominantly slender and somewhat pale green in stem colour with internodes up to about 6 cm. in length, whilst the latter are relatively short in stature with a noticeably darker green stem colour which often becomes purplish towards the base of the stem. Their internodes are short and the plant has a stocky appearance with a crowded leaf insertion. Notwithstanding the fact that the height of the winter plants is often only half that of the summer plants of similar age the number of internodes in both types at a similar stage of development is generally approximately the same. Where lateral branches are developed on the winter plants, these, too, are short and crowded and emphasise the stocky appearance of the plant.

For the purpose of this investigation collections were made of carefully graded series of plants of both summer and winter types. In practice it was found convenient to classify the plants under the following headings:

(i) *Seedlings*, i.e. plants in which the cotyledons were still fresh and in which the epicotyl had reached a length of 1-2 cm.

(ii) *Young plants*, i.e. plants showing some elongation of the epicotyl but in which the apical bud was still unexpanded and in which no flower-buds were visible. This group obviously includes plants of somewhat varying age.

(iii) *Plants with flower-buds*, i.e. with flower-buds showing, but few open flowers.

(iv) *Mature plants*, i.e. plants with fully expanded apical region and fruits in various stages of maturation.

TECHNIQUE

The layer of cells which gives rise to the endodermis can be distinguished at an early stage immediately outside the pericycle. The cells forming this layer are somewhat larger than the inner cortical cells or those of the pericycle. On making the usual tests it was found that this layer is a well-defined starch sheath.

Serial microtome sections, stained with a very dilute solution of aqueous gentian violet, were used for examination of the endodermal condition of the seedling.

Individual plants older than the seedling stage were examined by means of fairly close series of hand sections. Experiments were made with several stains for these plants. Among these were: (i) glycerin-alcohol Sudan III; (ii) glycerin-alcohol Sharlach R (both these being

modifications devised by Priestley for work of this kind); (iii) chlor-zinc iodine (using Artschwager's formula); (iv) iodine solution followed by sulphuric acid (in the proportion 2:1). After an extended trial, the last method was adopted as a routine procedure for the main part of the work. This stain gives the following results: the cuticle, Casparian strips and suberin lamella are stained brown, the xylem, slightly swollen, becomes yellowish brown. Cellulose walls become blue and much swollen, starch blue-black, and cytoplasm yellowish brown. Duplicate sections were mounted in gentian violet glycerin jelly to give permanent preparations. The use of this stain gives preparations in which the Casparian strips and the suberin lamella are clearly differentiated in bright purple. The xylem and other lignified tissues assume a somewhat paler tint, while the general mass of the ground tissue is only lightly stained.

THE CHARACTER AND DISTRIBUTION OF THE ENDODERMIS
IN THE PRIMARY STEM AND ROOT

As stated above, a starch sheath makes its appearance early in the life of the plant, being found within a few centimetres of the apex of the stem. The endodermal ducts, which will be described later, arise at this stage as intercellular spaces (Fig. 1*a*). In plants where true endodermis is developed rapidly the radial walls soon begin to acquire a Casparian thickening. By the time the Casparian strips are fully formed the starch has almost completely disappeared and the endodermis is in the "primary" condition (Fig. 1*b*). As time goes on the Casparian thickenings extend (Figs. 1*c*, 2*a*, *b*) until they cover the whole of the radial wall and afterwards an even deposit of similar material appears on the tangential walls so that the whole cell is now thickened and looks very different from a cell in the primary condition. At this stage, the "secondary" condition (Fig. 2*c*), no starch is present, though there is protoplasm in the cell, and division may occur with growth of the stele. No further development occurs in this plant into a "tertiary" condition.

The development of the endodermis in plants of different age and season of growth will now be considered.

(i) *Seedlings*

The youngest plants examined in detail were those in which the first two leaves were partly expanded, and in which the cotyledons were still fresh and green. The endodermis at this stage is wholly

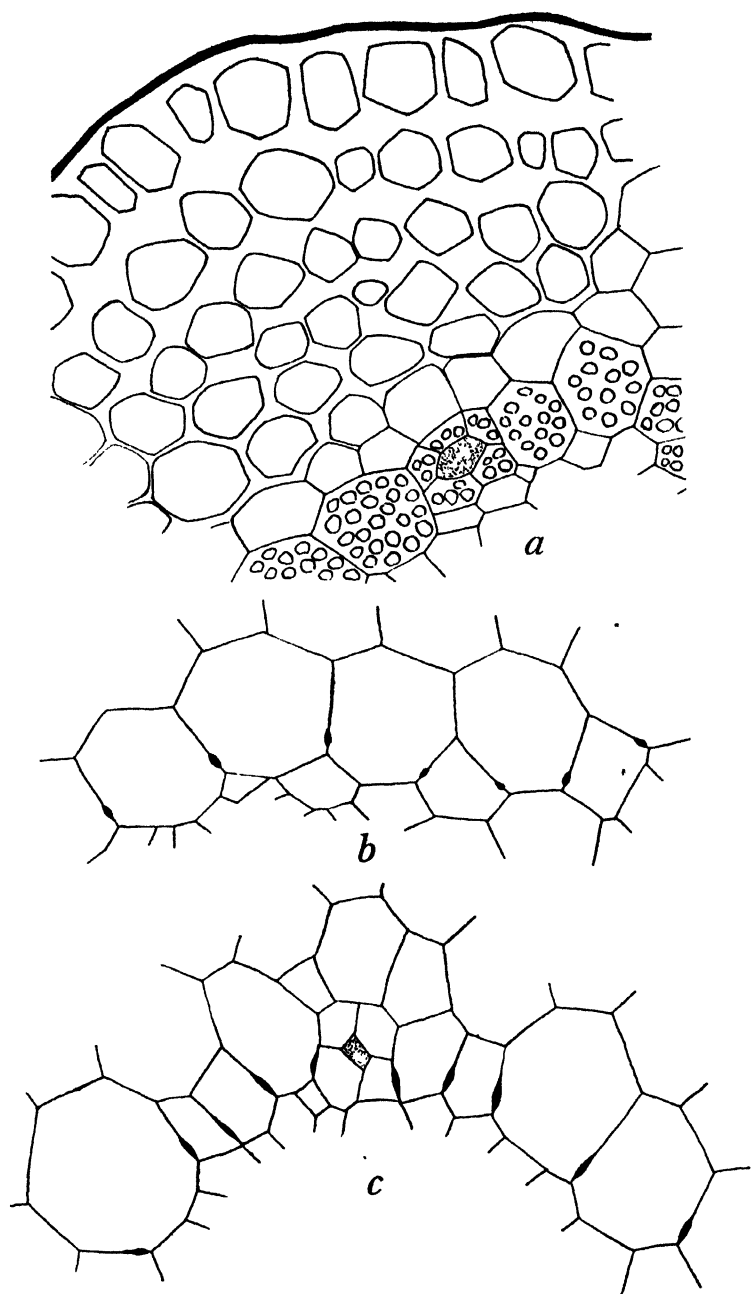


Fig. 1. Detailed drawings showing the typical development of the endodermis at various levels. *a*, The starch sheath in the peduncle. *b*, Early primary stage in the stem. *c*, At a lower level showing lengthening of the Casparian strips. (All $\times 433$.) Endodermal ducts are stippled in this and all subsequent drawings.

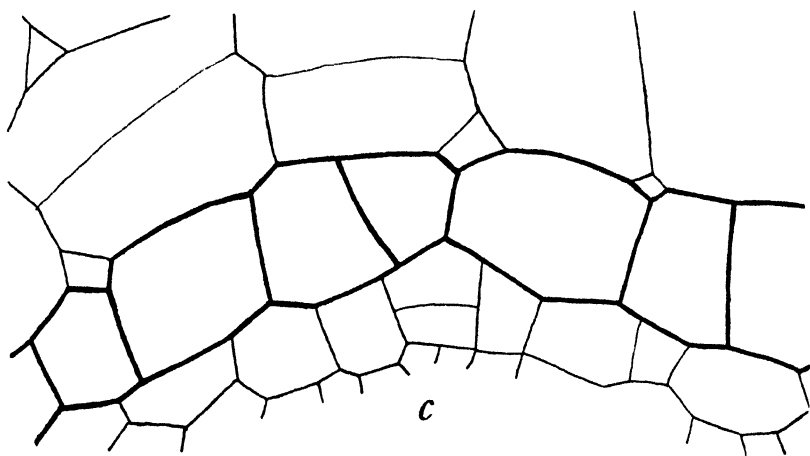
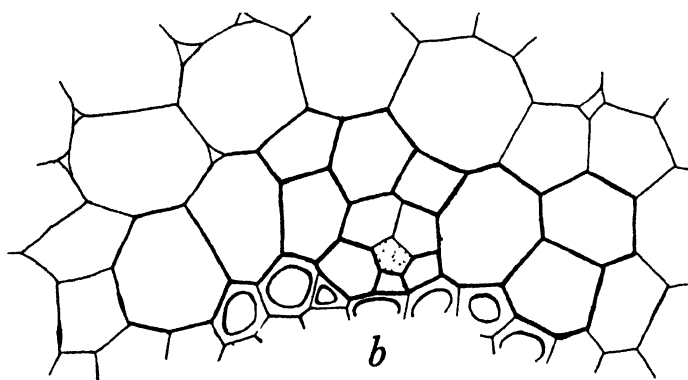
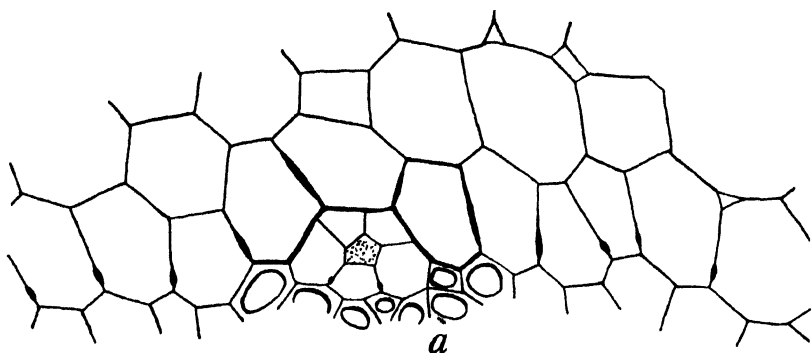


Fig. 2 *a-c*. Detailed drawings showing the subsequent development of the endodermis into the secondary condition, at lower levels in the same plant as Fig. 1 (all $\times 433$).

primary and extends into the epicotyl, in some cases into the second internode. No essential difference was noted in the endodermal condition of spring or autumn plants of this age. Fig. 3*a* shows the size and position of the Casparian strips at this stage. As the stele increases in girth the endodermal cells are stretched tangentially and subdivisions then occur in the parent cells. The majority of these division walls are radial, though in the hypocotyl and root tangential and oblique ones also occur. The radial division of endodermal cells with growth has been noted in other plants by Bond⁽²⁾ and Kroemer⁽³⁾ and is possibly of general occurrence. In *Senecio vulgaris* the radial walls and those which are somewhat oblique, soon develop a Casparian strip of quite normal appearance. No strips are developed on tangential division walls when these occur (Figs. 3*b*, 4*a*).

In the hypocotyl, where the xylem groups are uniting in the intercotyledonary plane, the endodermal cells which lie outside these xylem masses show distinctive features. They are characterised by a greater number of division walls than the remainder of the endodermal cells, and these division walls are in various planes (Fig. 4*a*). There is always one well-marked tangential division through each of the cells of the cap, this separating the layer into two rows of approximately equal size (Figs. 2*c*, 4*a*). Radial division walls also occur in the majority of the cells, and often some oblique ones in addition (Fig. 4*a*). The tangential and radial walls, when both occur simultaneously in the same cell, give it the appearance of having been divided into four parts. In one, or more frequently two, of these cells, at the intersection of the division walls, characteristic intercellular spaces are developed (Fig. 4*a-c*). From their appearance and general characteristics it seems clear that these intercellular spaces are secretory ducts similar in character to those occurring in the developed epicotyl and to which further reference will be made.

(ii) *Young plants*

The plants included in this group are those in which there is some elongation of the epicotyl, but in which the apical region is still unexpanded and in which no flower buds are visible. In this category come plants of somewhat varying age and size. The summer-grown plants and the winter ones differ in many respects and will be described separately.

(a) *Young summer plants.*

These plants are a fresh green colour, and give the appearance of having grown fairly rapidly. In the lower part of the epicotyl, inter-

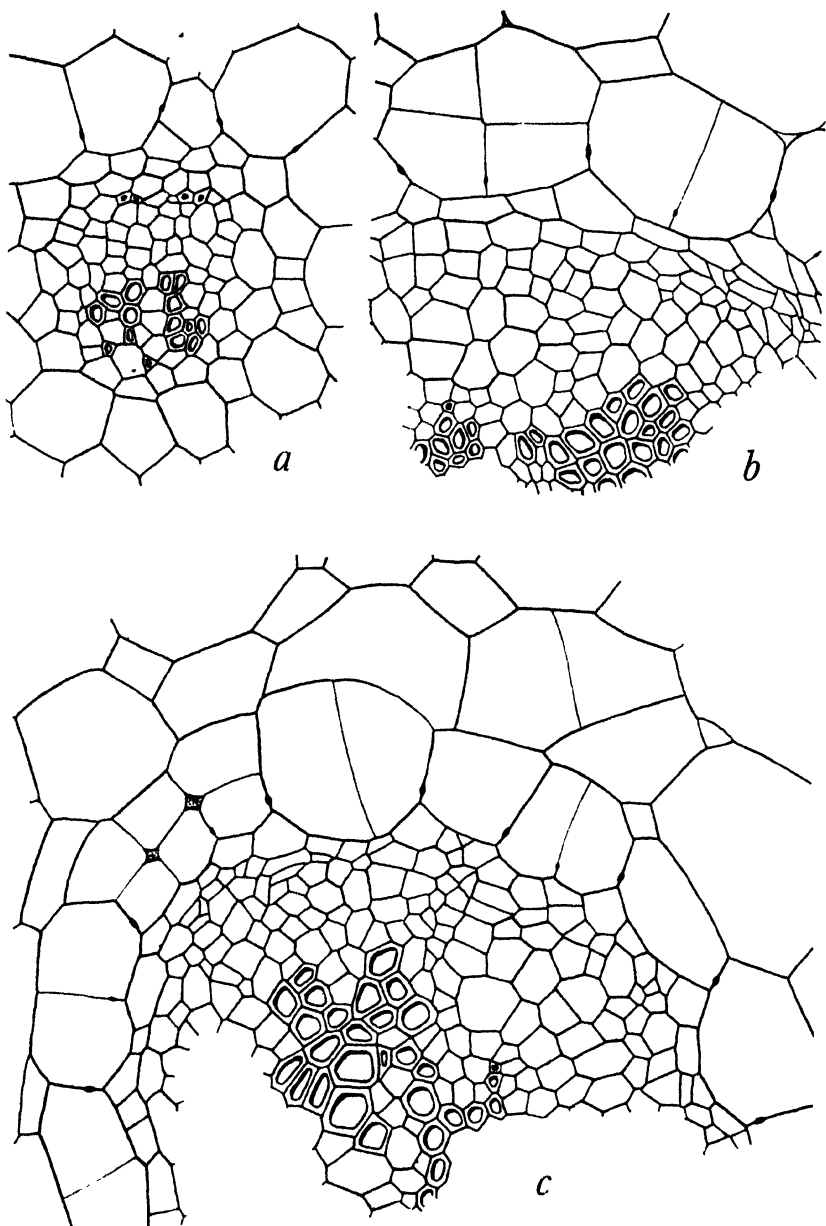


Fig. 3. Detailed drawings showing the endodermis of a seedling. *a*, Young vascular bundle with associated primary endodermis. *b*, Part of upper hypocotyl showing subdivision of endodermal cells and development of Casparian strips on the new radial walls. *c*, Lower region of hypocotyl showing development of two ducts in the intercotyledonary plane. (All $\times 216$.)

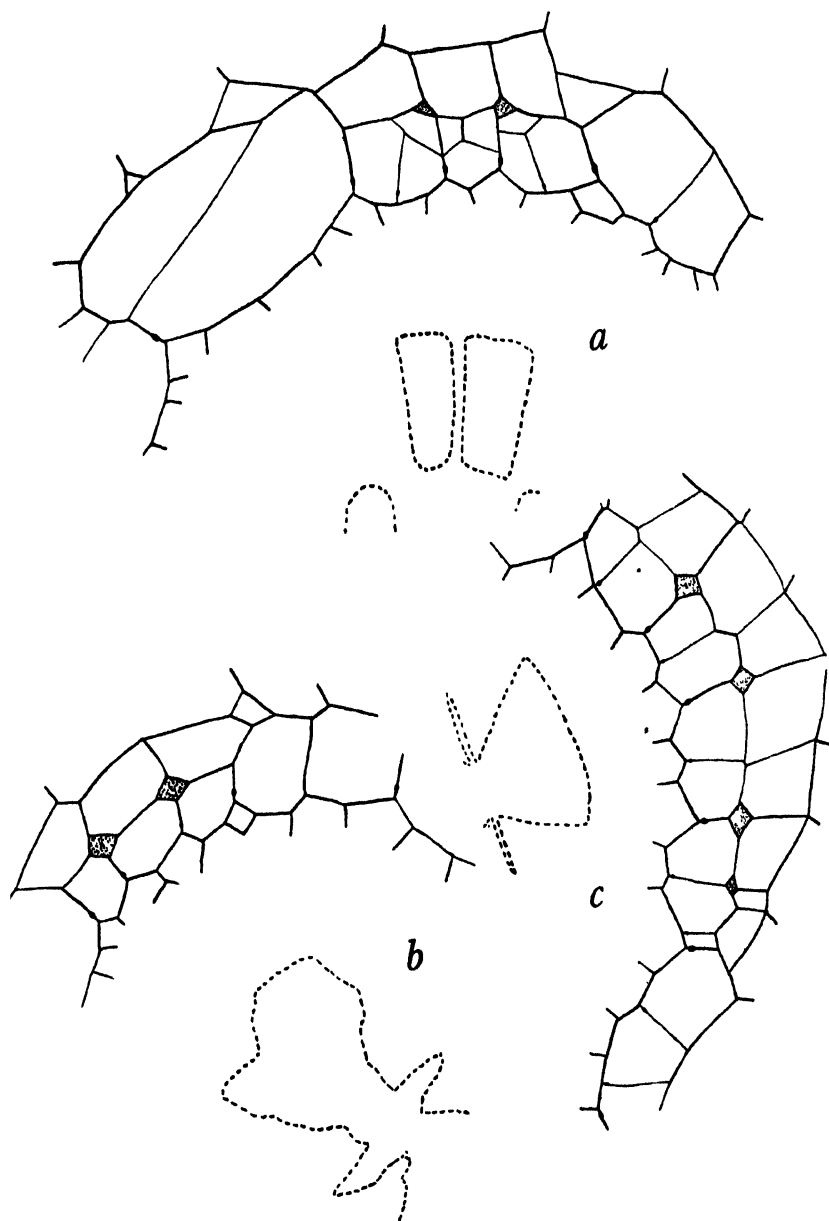


Fig. 4 *a-c*. Detailed drawings showing varying duct conditions in the hypocotyl of the seedling (all $\times 216$). Outline of xylem indicated by dotted line.

facicular cambium is seen between the vascular bundles, though it has given rise to no secondary xylem. A primary endodermis is always present in the first internode and in most cases extends into the lower part of the second, but is absent from all the upper internodes, where it is replaced by a well-defined starch sheath. The first appearance of the endodermis in the stem is always discontinuous. Casparian strips appear in groups of localised cells separated by series of unmodified cells (Figs. 5*a*, 6*a*). The most usual position for these groups of strips is in caps over the bundles (cf. Bond (1), (2)). Ducts similar in character to those found in the hypocotyl of the seedling are found outside some of the bundles (Figs. 5*a*, 6*a*). In successive sections and below the level at which Casparian strips are first recognised, an increasing number of cells is found to have formed one (Fig. 5*b*), until in some cases they form a continuous arc over several adjacent bundles. This development, however, is markedly irregular in its incidence, so that at the same level a whole series of bundles may be capped by a continuous primary endodermis, whilst on the opposite side of the stem it still occurs over isolated bundles (Fig. 6*a*). The endodermis is always continuous in these plants just above the cotyledons. The ducts mentioned above are found to be fewer in number at the base of the stem than at the apex, and they are frequently absent in the upper part of the hypocotyl. They are found again towards the base of the hypocotyl in the groups of cells in the intercotyledonary plane. Their appearance in this position is consistent in *all* types of plants examined (Fig. 7). The cells in the cotyledonary plane are characterised by numerous cell divisions but in no case are ducts found in this region (Fig. 5*c*). The endodermis is continuous in the hypocotyl and root. It is still primary in the hypocotyl, but in some cases in the older parts of the root a few isolated cells are found which have passed into the secondary endodermal condition.

A slightly larger, and no doubt, older summer plant (intermediate between the typical "young" plants of this group and those of the "flower-bud" group) was examined. In this plant a little secondary xylem is found between the bundles in the first internode, and pericyclic fibres are just beginning to be formed. Its endodermis is more advanced; primary endodermis is developed in the second internode and is continuous throughout the first internode. Just above the cotyledons a very few of the endodermal cells are in the secondary condition, in some places a single cell being in this condition, in others two or three together. In the hypocotyl and root more

secondary endodermal cells are found, but nowhere is it continuous, and in the intercotyledonary plane the endodermis always remains in the primary condition.

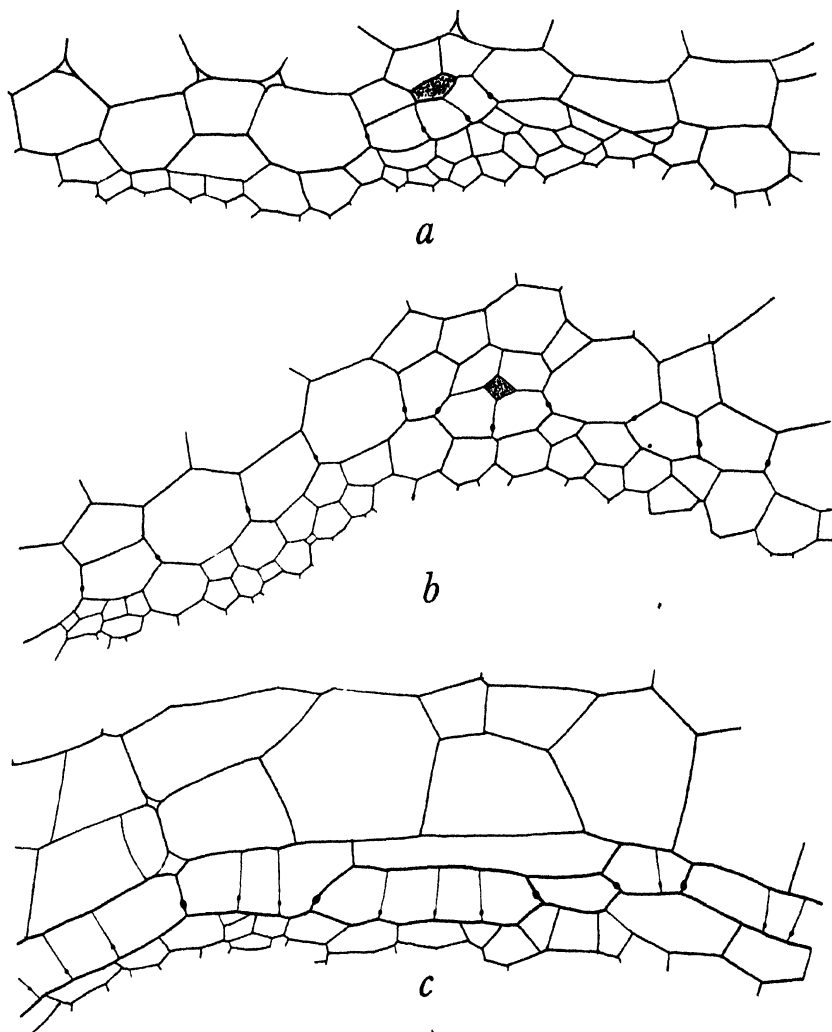


Fig. 5. Detailed drawings showing the character of the endodermis in the main axis of a young summer plant, taken from above downwards. *a*, First appearance of Casparian strips in the stem opposite a vascular bundle. *b*, Continuous primary stage in stem. *c*, Endodermal cells in primary stage with numerous subdivisions in the cotyledonary plane of the hypocotyl. (All $\times 233$.)

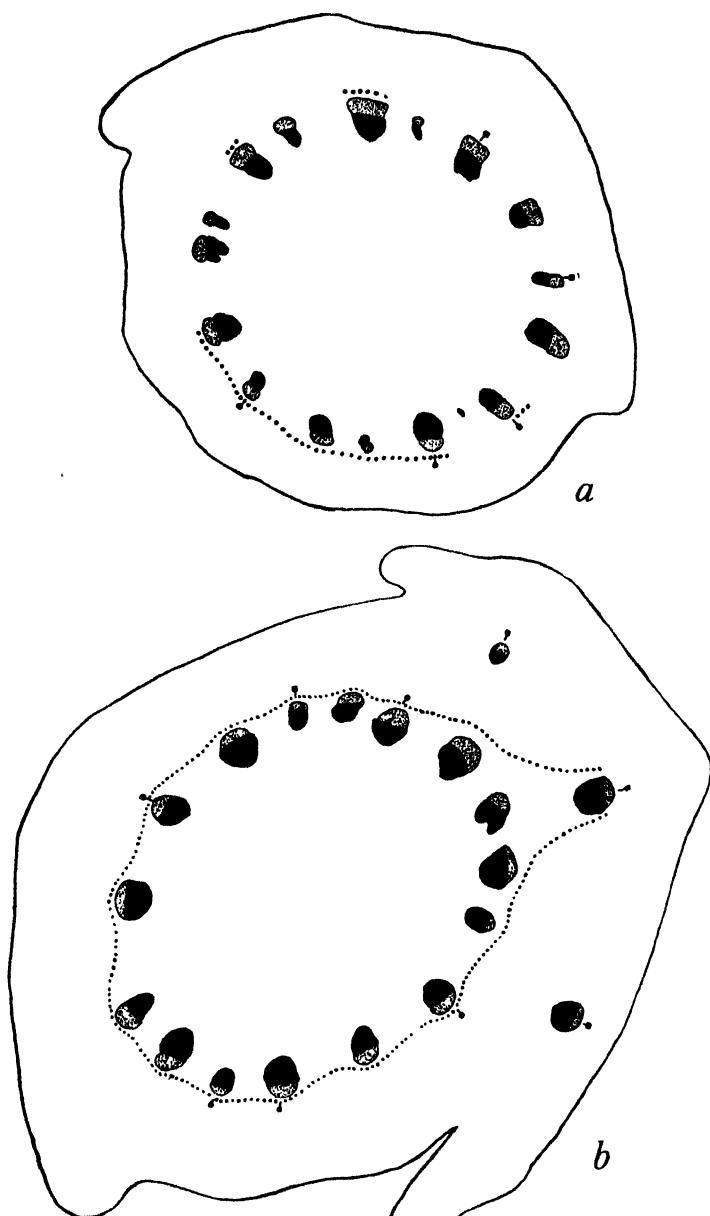


Fig. 6. *a*, Camera lucida outline of the stem of a young summer plant showing the development of the primary endodermis. Xylem indicated by solid black, phloem by stippling, primary endodermis by dotted line, ducts by small black squares with a tail. (These conventions are used in all subsequent outline drawings.) *b*, Camera lucida outline of the stem of a winter plant with flower buds showing continuous primary endodermis and its behaviour with reference to the leaf-trace bundles. (Both $\times 25$.)

(b) *Young winter plants.*

The stem and leaves of these plants are darker green than those of the summer-grown plants, and the lower part of the axis is usually purplish or dark brown in colour. The internodes are short, so that although the total number of nodes is greater than those of the summer plants at this stage, the average height is approximately the same and the whole plant has a stocky appearance. In all the plants examined the lower leaves, that is about half the total number, including the cotyledons, are dead or dying.

The anatomical structure of the plants shows evidence of greater maturity than the summer plants. Interfascicular cambium occurs in the middle internodes of the axis and in most of the plants examined secondary xylem is developed in the lower internodes and is continuous through the hypocotyl. In some examples the outer cells of the pith are lignified and pericyclic fibres are found outside some of the vascular bundles. No correlation could be established between the condition of the endodermis and the development of secondary elements.

The endodermis in all cases extends into the upper two-thirds of the axis, appearing in one plant in the second expanded internode below the apical region. Its origin and development is similar to that of the young summer plants, but in all these winter plants the secondary condition is found in the lower part of the epicotyl and extends in most cases through nearly half the total number of internodes (Figs. 8, 9). It is interesting to note in this connection that the level at which secondary endodermal cells are first seen coincides with that where the leaves are dying. In most cases there is a considerable number of cells in the secondary condition throughout the hypocotyl and the base of the root. Their distribution in the main root is irregular, though the number of them decreases towards the apex of the root.

The groups of cells in the intercotyledonary plane to which reference has been made in the seedling and young summer plants, are always the last to develop the secondary condition, remaining primary in many cases when the rest of the endodermis is almost wholly secondary (Fig. 7).

Division walls also occur in endodermal cells in the secondary condition, and it is worthy of note that *a Casparian strip is never found on a division wall in any plane of a cell already in this stage of development.* The division walls either persist as delicate radial plates of cellulose, or they develop a suberin lamella (Figs. 7 and 9).

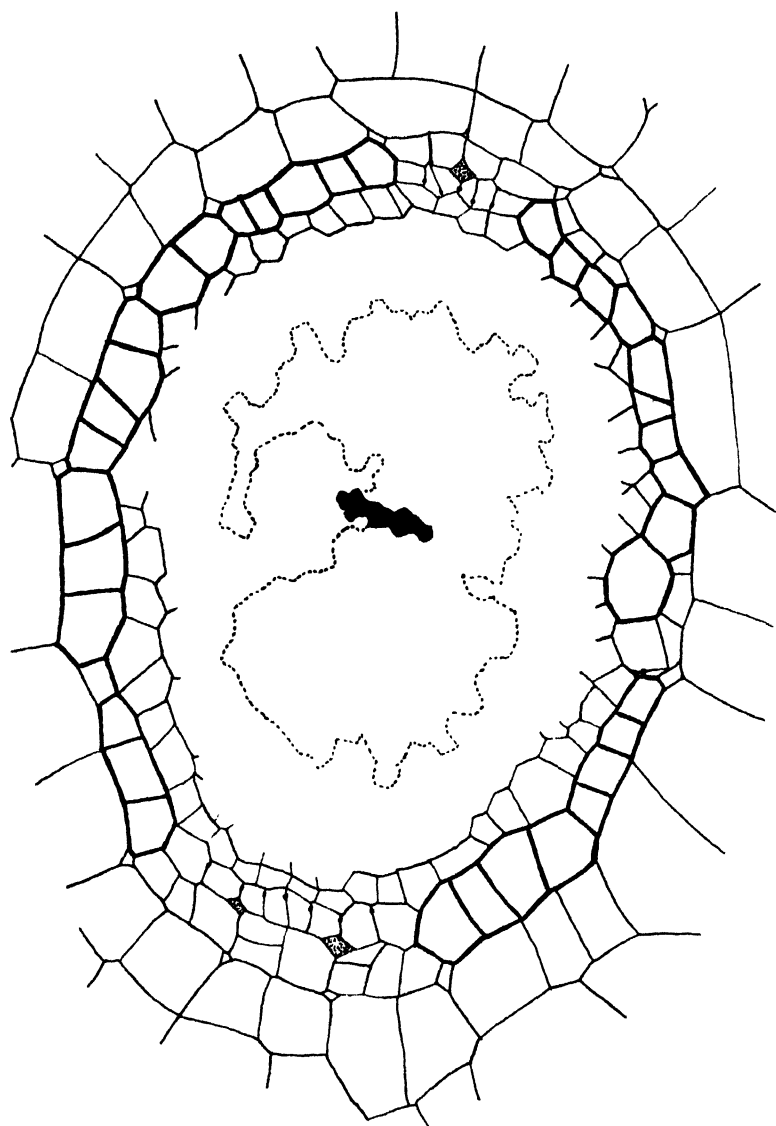


Fig. 7. Transverse section of the stelar region of the primary root of a young winter plant showing persistent caps of primary endodermis in the intercotyledonary plane, and division of endodermal cells in the secondary condition. Primary xylem indicated by solid black, area of secondary xylem by dotted line ($\times 233$).

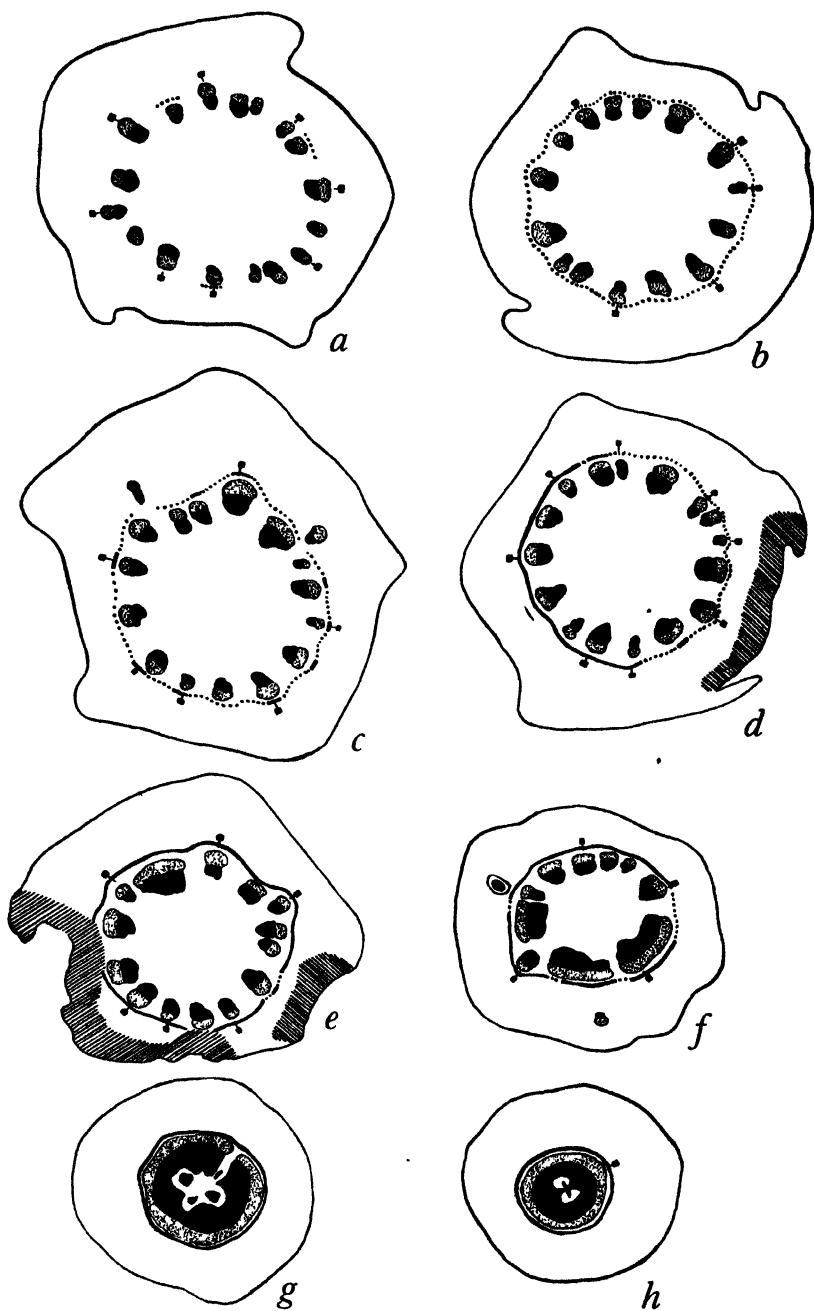


Fig. 8 *a-h*. Camera lucida outlines of the main axis of a young winter plant showing the distribution of primary and secondary endodermis (all $\times 32$). Secondary endodermis indicated by heavy continuous line. Shaded area in *d*, *e* indicates wound tissue.

It will be seen from the above description that the vertical extension and degree of maturity of the endodermis is considerably greater in winter plants than in summer plants in a similar condition with regard to flowering. This difference is expressed diagrammatically in Fig. 13.

(iii) *Plants with flower-buds*

These plants (and also those of the next group with open flowers) are of somewhat more diverse age and size than the "Young Plants", but the same broad distinction can be made between the characteristics of summer and winter plants in which the flower-buds have attained approximately the same stage of development, and a general difference in endodermal condition is still to be found.

(a) *Summer plants with flower-buds.*

This group includes some plants which in size and from an examination of their anatomical structure seem to be little more advanced than those described in the previous section, whilst others are much more developed in height and girth, and show cambial activity in the lower part of the axis. The number of internodes varies from six to ten. It is interesting to note that the endodermis is found only in the lowest two internodes and that there is very little difference in vertical distribution, and no difference in the character of endodermal cell between the young and more developed types.

(b) *Winter plants with flower-buds.*

In the November-February plants with flower-buds a continuous layer of primary endodermis is found two-thirds of the way up the stem, and discontinuous stretches of it occur in the next internode. In the root, hypocotyl and lower internodes the endodermis is partly in the secondary condition, and the correlation between the occurrence of secondary endodermal cells and the dying off of leaves still exists.

Secondary endodermal cells are first found opposite certain of the vascular bundles and increase in numbers towards the level of the cotyledons. Below this level a variation in their development is found in different plants. In some they increase in the hypocotyl until in the root a continuous secondary endodermis is found, while in others they decrease and sometimes disappear in the hypocotyl, occurring again at the base of the root just below the region where it expands suddenly on joining the hypocotyl.

In the root the secondary endodermal cells are very much stretched tangentially and show numerous subdivisions; as before the division

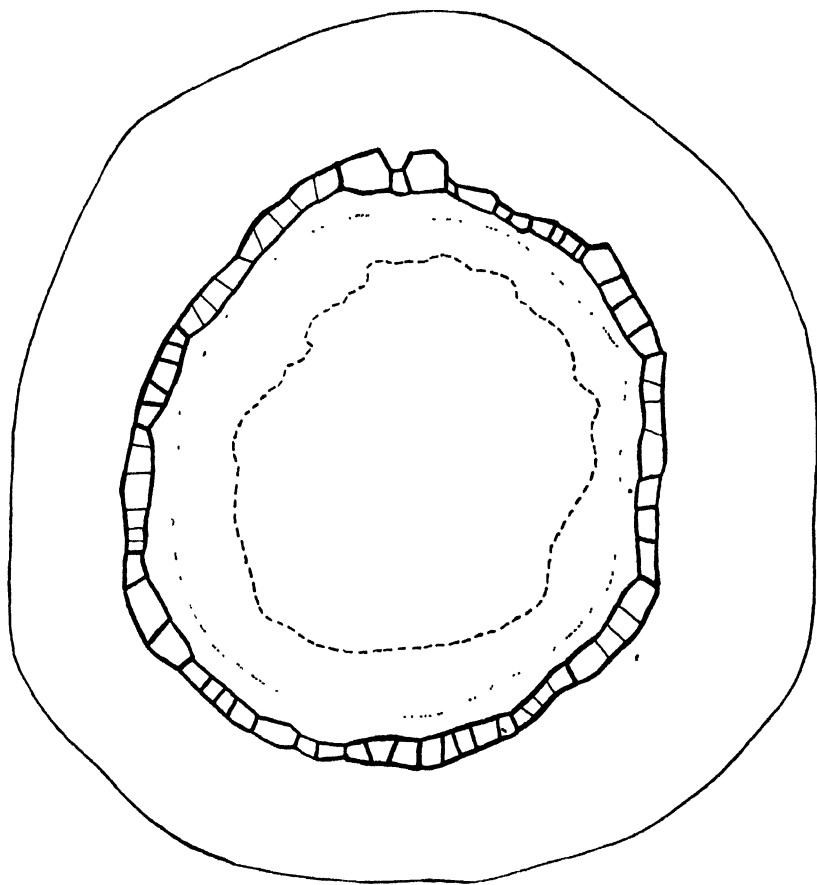


Fig. 9. Transverse sections of the base of the stem of a winter plant with flower buds showing a continuous secondary endodermis with numerous subdivisions in the endodermal cells. *Note.* Subdivisions either possess walls thickened in similar manner to those of parent cells or are thin radial walls without deposit. Outline of phloem indicated by fine dotted line, outline of xylem by broken line ($\times 433$).

walls either possess a suberin lamella like the parent cell or are free from deposit. As with the "young plants" there is thus a great difference in the extent of the endodermis in the summer and winter plants. This is summarised diagrammatically in Fig. 13.

(iv) *Mature plants in flower*

Typical plants in this group are well developed, possessing twelve or more internodes and bearing mature plants and fruits. Lateral shoots are borne in the axils of most of the leaves. Plants of this age and development were gathered in July, September, October, December and February. The difference in the vertical extension of the endodermis noted in the summer and winter plants at a younger age is *found to be non-existent in these fully mature plants*. It is unnecessary, therefore, to describe them separately.

An endodermis is found to extend throughout nearly the whole main axis. The highest level at which it is found in the primary condition is in the internode below the peduncle, and in the next internode it is usually continuous. About this level secondary endodermal cells are present. (In rapidly grown summer plants certain cells may reach the secondary condition even before the primary endodermis is continuous (Fig. 10*a*).) Below this the proportion of secondary cells steadily increases (Fig. 10*b*) until the hypocotyl is reached, where as in the plants with flower-buds it may either increase or decrease. Even when it decreases in this region it increases again in the main root and a continuous secondary endodermis is found in its narrow part.

CHARACTER AND DISTRIBUTION OF THE ENDODERMIS
IN LATERAL ORGANS

The leaves, lateral stems and roots of plants of various ages were investigated, but it will be possible to base the description of their endodermis in fairly mature plants with only occasional reference to its development in younger plants.

Leaves. As the leaf-trace bundles emerge from the stele and enter the petiole they lose their endodermis (Figs. 6*b*, 8*c*). Neither endodermis nor starch sheath is found in the leaves of this plant. In the deeper layers of the cortex the leaf-trace bundles may possess a secondary endodermis (Fig. 8*f*) in winter plants, but the condition of the endodermis surrounding these bundles is usually in a younger state than that of the neighbouring bundles of the stele.

Lateral shoots. In these an endodermis is frequently found. Its origin and increase in number of modified cells is exactly similar to that described in the main stem, but a short account of its distribution is necessary. The larger lateral shoots possess three or more internodes. The endodermis is usually confined to the lower internodes

where its condition suggests a fairly rapid development similar to that near the apex of the main stem. Where the endodermis is just

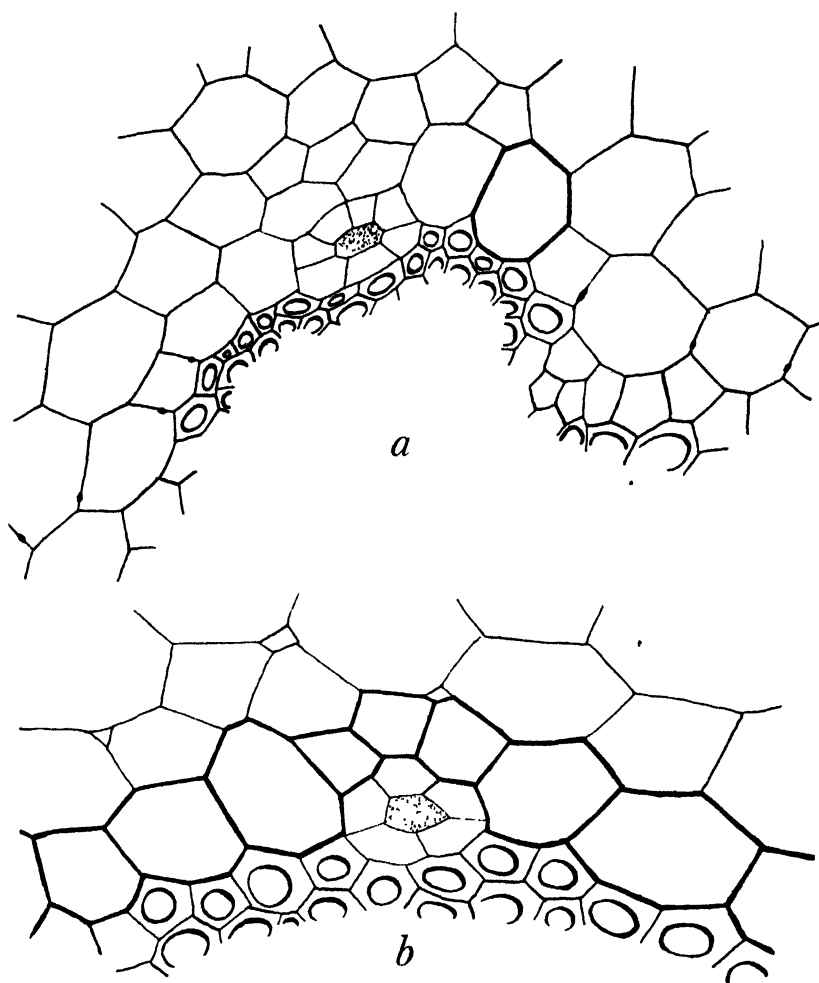


Fig 10. *a*, Detailed drawing of transverse section of the stem of a mature plant showing the development of a secondary endodermal cell before the primary endodermis is continuous. *b*, Detailed drawing showing the endodermis in the continuous secondary stage at a lower level in the same plant, and its relation to the duct. (Both $\times 433$.)

beginning to develop secondary cells in the main axis the base of the lateral is found to possess a continuous primary endodermis; where the secondary condition is continuous in the main axis it shows the

characteristic primary condition with many cells bearing elongated Casparian strips and some, opposite the vascular bundles, in the secondary condition.

Lateral roots. These are relatively stout. They have a very consistent type of endodermal development. A continuous primary endodermis is found near the apex (Fig. 11*a*), but even here a difference can be observed between the cells opposite the xylem and

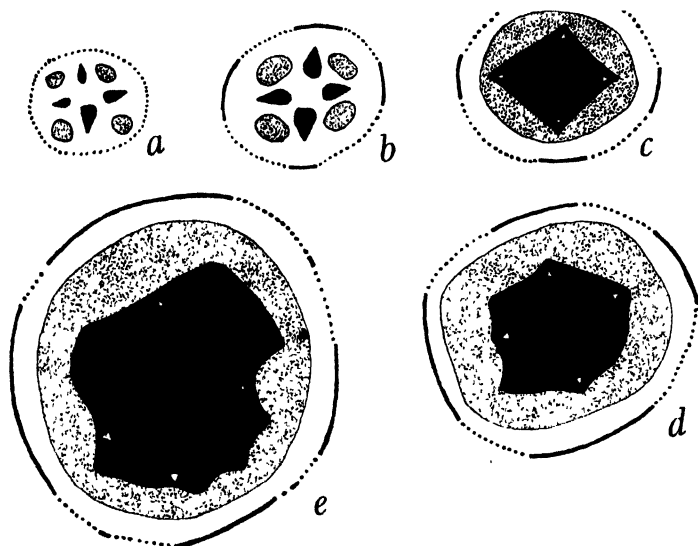


Fig. 11 *a-e* Camera lucida outlines of a series of transverse sections of the stele of a lateral root of a mature plant showing the distribution of primary and secondary endodermis from the apex to the base. Note. The secondary endodermis is initiated opposite the protoxylem groups (all $\times 70$).

those opposite the phloem; those opposite the phloem show divisions in radial and tangential planes and the development of intercellular ducts (Fig. 12*b*). Slightly further back, but before the groups of xylem have become continuous, the cells opposite the protoxylem are in the secondary condition (Figs. 11*b, c*, 12*a, c*), and successively nearer the point of origin the secondary endodermis becomes more extensive (Fig. 11*c-e*). In the oldest lateral roots it may become continuously secondary, but much more commonly some of the cells opposite the original phloem groups remain in the primary condition.

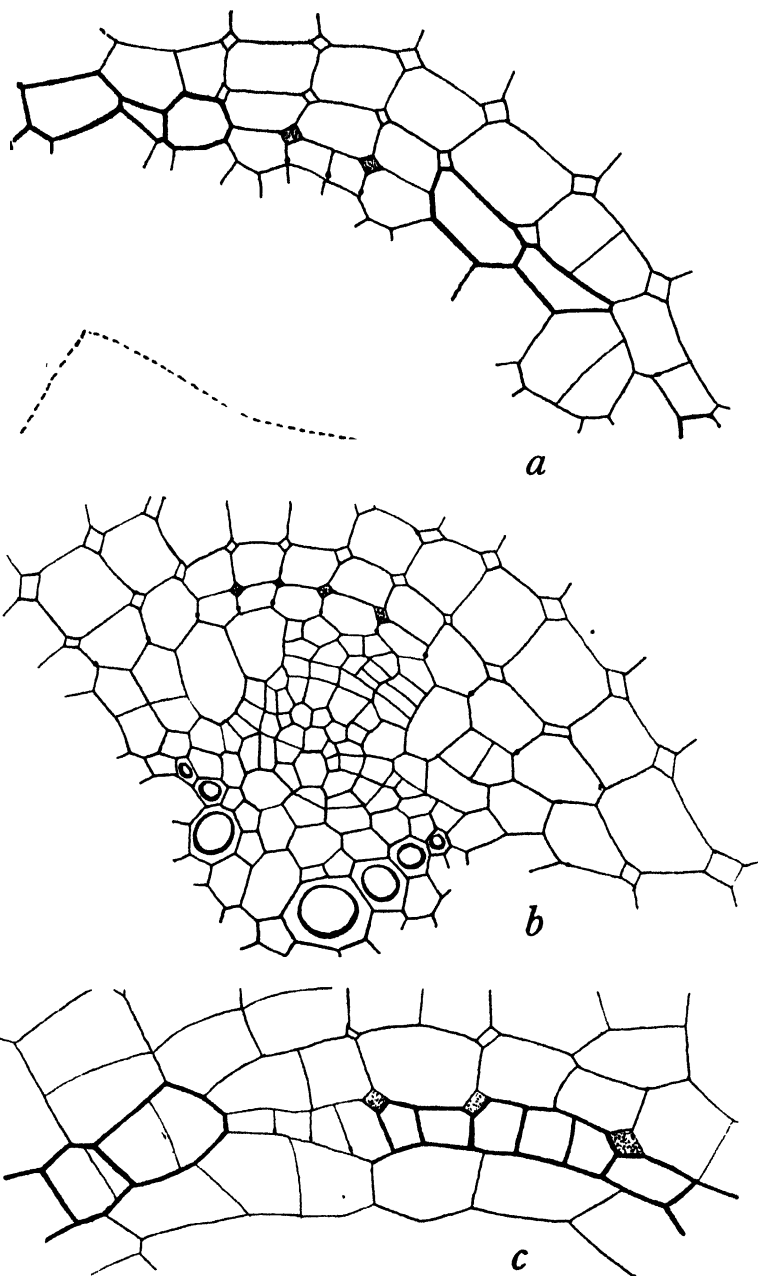


Fig. 12. Transverse sections of lateral roots of mature plants. *a*, Showing endodermis and ducts near the point of origin of the lateral root. *b*, The same near its apex. *c*, Endodermis of an older lateral root, showing the occurrence of secondary cells and ducts which are opposite the phloem groups. (All $\times 433$.)

SECRETORY DUCTS ASSOCIATED WITH THE ENDODERMIS

The ducts, to which some references have already been made, are initiated in the growing plant in association with the starch sheath at a very early stage of development. They can be detected in sections of the seedling opposite some of the vascular bundles when these are only just beginning to be differentiated. No new ducts are ever initiated at any distance from the growing apex of the stem.

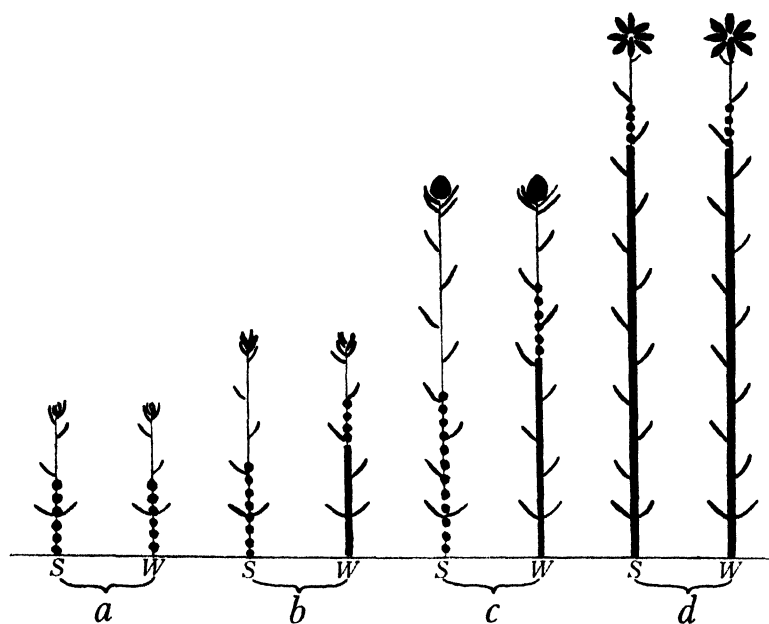


Fig. 13. Diagram to illustrate the extent of the endodermis in plants of different age and season *a*, seedling plants, *b*, young plants; *c*, plants with flower buds; *d*, mature plants in flower. *S*, indicates summer plants; *W*, winter plants of corresponding age. Primary endodermis indicated by dotted line, secondary endodermis by heavy black line.

are found both in the mid-rib and the main lateral veins of the leaf, occurring in a position opposite the bundles as in the stem, though here neither starch sheath nor endodermis differentiate. They accompany these bundles into the stem, though from an examination of serial sections it appears that many of them disappear after a distance of one or two internodes after their entry into the stem. In the lower part of the stem they only extend a short way below a node,

so that in the lower internodes they are fewer in number than in the middle internodes, in some cases only two or three being present in the first internode. Although their normal position is in the centre of the arc of cells opposite the vascular bundles they are sometimes found at one side of this arc, notably in the peduncle, and in the main stem they may be situated at one side of this arc of cells where two bundles, one possessing a duct, have fused. In rare cases two ducts are found outside one bundle, but where this is the case they usually fuse later to form one. As a rule they are absent in the upper part of the hypocotyl, but one or two are always found in the groups of endodermal cells in the intercotyledonary plane at a lower region in the hypocotyl. In the main root they were not found, but in the lateral roots they occur in a very regular manner in rows opposite the phloem (Fig. 12 *a-c*). Their position and origin is similar to that described by Von Triebel(11) in his account of oil ducts in the roots of several species of the Compositae. No evidence is found, however, in *Senecio vulgaris* of the displacement of these ducts into the cortex. They remain small and associated with the endodermal layer.

Although the ducts are formed in close association with the starch sheath, the exact incidence of primary and secondary endodermal characters on the adjacent cells is surprisingly varied. Almost all possible relations are found and several are figured (Figs. 1 *b*, 2 *a, b*, 5 *a, b*, 10 *a, b*), the commonest condition being for the cells forming the inner side of the duct to possess endodermal characters corresponding with the rest of the endodermis (Fig. 1 *c*, 2 *b*, 5 *b*).

DISCUSSION

We now possess a considerable body of data concerning the occurrence of the endodermis in vascular plants in general and in the stems of land plants in particular. We are, however, largely ignorant of the causes which lead to its development and of its function when present. Widely divergent views have been expressed by Priestley(5-9) and Bond(2), but a discussion of these views does not fall within the scope of this paper.

In *Senecio vulgaris* the endodermis develops in the seedling and in all cases is found in the developing axis, but in plants growing under summer conditions this development is slow until the onset of flowering when it proceeds rapidly in the main axis, and in lateral shoots, where its initiation is naturally late; its development when flowering begins is correspondingly rapid.

It is noteworthy that the endodermal condition has no relation to the actual size of the plant, but only to its maturity. This was borne out by the investigation of a number of depauperate specimens collected from slag heaps, clay heaps, and sand heaps which were compared with plants grown in cultivated soil. These facts suggest that some feature exists in the metabolism of the plant at the flowering stage which accelerates the development of the endodermis.

The discovery of a difference between plants growing in the winter months in which the extent of the endodermis is considerable, even in young plants, and is *noticeably* greater than in summer plants of corresponding age, suggests that there is an additional factor at work accelerating endodermal development at this season. As an explanation of the earlier development of the endodermis in the winter plants it may be suggested that under cold conditions fat is produced at the expense of carbohydrates. It should be noted that several investigators⁽¹⁾ have shown that many plants partly or wholly replace carbohydrates by fatty material during the winter periods.

Another possibility is that the difference is due, not to cold, but to the shorter period of illumination which has a profound effect on many plants (cf. Tincker⁽¹⁰⁾). This was, however, definitely disproved to be the cause of the difference in this case. Parallel sowings of seeds from summer plants were made in the early summer in adjacent plots, and one batch was left to grow without interference whilst the other was covered so that it was only illuminated for 8 hours daily (from 9 a.m. to 5 p.m.). The contrast between the two batches was striking; those exposed to the shortened period of illumination being darker in colour and stocky in character and thus resembling typical winter plants, the others being relatively tall with well-developed internodes. In both cases, however, the distribution of the endodermis was that typical of normal summer plants.

An attempt was made to find out what effect could be caused by cold on summer-grown plants, by growing plants in a cooled chamber, but owing to the inadequacy of the apparatus, no result at all was obtained. It is hoped that further work on these lines will be possible.

The following suggestions are made to explain the various phenomena of the development of the endodermis in this plant:

(1) We may suppose that there is a steady accumulation, with time, of fat in the starch sheath layer, and this is ultimately deposited, first as a Casparian strip and later as a "secondary" fatty lamella.

(2) Flowering causes an alteration in the metabolism of the plant

which accelerates this process, independently of season and of size and age of the plant, causing all but the very youngest cells in this layer to differentiate as endodermis.

(3) Under winter conditions fat accumulation is more rapid than in summer, and leads to earlier differentiation of primary and secondary endodermis. Possibly this accumulation is influenced by the death of the leaves.

It is suggested, therefore, that there is a steady drift towards endodermal development with two independent factors which may operate to accelerate it.

SUMMARY

1. An endodermis is present throughout the main root, the lateral roots, and to a varying extent in the stem and the lateral shoots of *Senecio vulgaris*. It does not occur in the peduncle or leaves. Both primary and secondary endodermal cells are found.

2. The distribution of the endodermis and the character of the endodermal cells varies in plants maturing under different physiological conditions, those maturing under winter conditions possess an endodermis which extends to a much greater height in the stem than those maturing under summer conditions, and the winter plant develops more secondary endodermal cells throughout the axis than the summer plant. At maturity this seasonal difference is not shown, because of a rapid development of the endodermis at the time when the summer plant flowers.

3. There appears to be correlation between the initiation of the secondary condition and the dying off of the lower leaves of the shoot.

4. Increase in size of the vascular cylinder is accompanied by tangential stretching of the endodermal cells followed by cell divisions, the majority of which are radial. Casparian strips are developed on the radial division walls of these cells when the parent cells are in the primary condition. The division walls of cells in the secondary condition are either free from deposit or covered with a suberin lamella. Casparian strips are never developed on the division walls of cells in the secondary condition.

I wish to thank both Prof. H. S. Holden of Nottingham, who suggested this investigation, and Prof. T. M. Harris of Reading for their kindly help and criticism during its progress and completion.

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MECHANICAL STIMULATION AND RESPIRATION RATE IN THE CHERRY LAUREL

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(With 12 figures in the text)

INTRODUCTION

THE results of various isolated investigations have all shown that a marked increase in the respiration of plant tissues can be brought about by wounding. Boehm (1887) seems to have been the first to have noticed an effect of wounding on plant tissues. He discovered that peeling and cutting up potatoes increased their respiration, which reached a maximum, in general, at about 36 hours from cutting. The fermentation of these tissues in hydrogen was not affected by the cutting. This work was followed by that of Stich (1891), who, besides experimenting on various seedlings, rhizomes and leaves, investigated the effect on the respiration of sealing together the cut surfaces of the potato tuber. The halves were bound together and the wound edges sealed with neutral gelatine. Although he confirms Boehm's results for cutting, his experiments on resealing were of too short duration to warrant any conclusions being drawn from them. This work was repeated in 1896 by Richards, who took continuous readings of the respiration of the cut potato, and thus obtained smooth curves of the effects of cutting. From the CO_2 -output drifts he discovered two effects of this cutting. Firstly there was an initial and sudden outburst of CO_2 due to the rapid diffusion outwards of the excess CO_2 in the intercellular spaces of the tuber and in the cells themselves. Secondly there was a real wound effect showing a much more gradual rise in the respiration, which reached a maximum after about a day. This latter effect he correlated with the formation of cork tissue healing the wound. Since the work of Richards very little has appeared on the subject. Lutman (1926) in America found that he could repeat Richards' experiments, but his technique was so inferior to that of Richards that nothing of importance comes from his work. He obtained indications of a rise in the reducing sugar content of his tubers after repeated cutting. He regards

the rise in the respiration as running parallel with the rise in the reducing sugar, which itself is bound up with the formation of the callus in the healing of the wound. This increase in the sugar content was also observed by Hopkins (1927), who also held that it was due to activities leading up to callus formation.

As a result of these researches the various authors conclude that the actual cutting of the cells gave rise to a change or changes in the cells around the cut resulting in the increase in the respiration. Thus the stimulus causing the observed effects would be a real wound stimulus. During the course of investigations on the starvation drift of the respiration in mature leaves of the cherry laurel an effect on the respiration due to a completely different type of mechanical stimulation has been noticed. It was discovered that if, during the course of respiration experiments on starved leaves in the dark, leaves were taken out of the chamber and then replaced, the subsequent respiration showed a much greater value after the procedure than before it. Experiments have shown that this increase in the respiration, which may be a rise to over twice the normal drift rate, is due to handling alone. The stimulation causing this increase consists solely of bending or rubbing the leaf, which is in no way damaged by the process. Thus here we are not dealing with a wound stimulation, as has been observed in cutting bulky storage tissues such as the potato, but with a stimulus arising from a mere deformation of the cells of the leaf during the bending or rubbing. It is conceivable that this type of stimulation might have entered to some extent into the wound stimulation in the experiments of the above-mentioned authors.

METHODS OF EXPERIMENTATION

The material used in the experiments to be described was mature leaves from five similar bushes which had been grown side by side from cuttings all from the same parent bush. The samples of leaves for each experiment were all carefully chosen from the same position on the bush, and as near as possible from the same position on the branches of the current year. Only leaves of the current year were used. Leaves were as a rule gathered in the afternoon, brought back to the laboratory and sealed into their respiration chambers within an hour from plucking. The type of respiration chamber used is shown in Fig. 1. The chamber and trough are made of metal, the inside of the iron trough being coated with paraffin. The petioles of the leaves were cut smooth with a razor and placed in water in the

trough. The whole chamber was sealed by running luting wax round the top of the lid in a groove made for that purpose. The respiration was carried out at a constant temperature of 22.5°C . Estimations of the respiration were by the Pettenkofer-tube method, using an air current

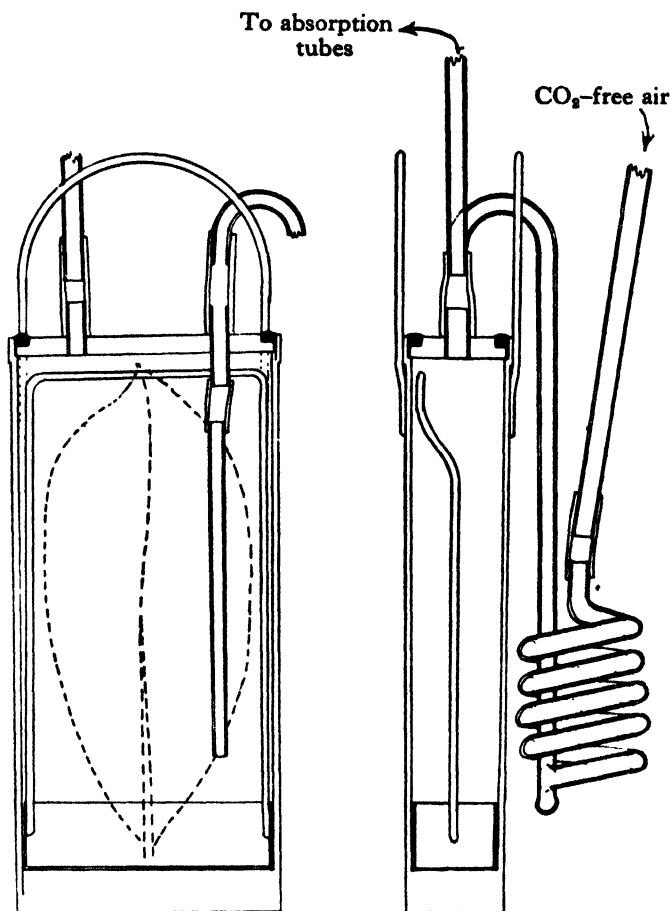


Fig. 1. The respiration chamber.

rate of approximately 2 litres per hour. Continuous 3-hourly readings of the respiration were obtained by making use of a series of Pettenkofer tubes and an automatic air current commutator for changing the gas stream from one tube to the next every 3 hours. In this way continuous readings of the respiration could be obtained over periods of many weeks. With these 3-hourly readings the chambers were emptied about twelve times for every reading, thus ensuring that

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in any rapid transitions no distortions would be produced owing to too long a lag between the evolution of the CO_2 from the leaf and its absorption in the Baryta. The air was taken from outside the laboratory and the CO_2 removed from it with soda-lime. Stepped graphs of the respiration rate against time were plotted, and smooth tracings of these graphs made. These two kinds of graph are seen in Figs. 6 and 7.

DISCOVERY AND CAUSES OF THE EFFECT

As previously mentioned, it was found that if leaves were removed from the chamber for a few minutes during the course of an experiment, the subsequent respiration on replacing them in the chamber

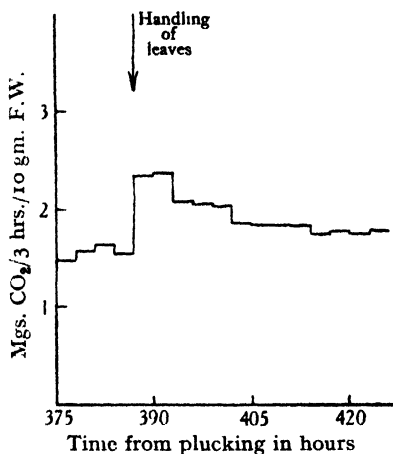


Fig. 2. Typical effect on the subsequent respiration of a sample of leaves of removing them from the respiration chamber and then replacing them. The leaves received very slight handling, but showed an increase in the respiration of the order of 60 per cent.

was considerably higher than that previous to opening the chamber. A typical result of this procedure is seen in Fig. 2. In a consideration of the possible causes of this rather striking effect it was realised that the process of removing leaves from the chamber involves the changing of a number of environmental conditions. Thus in taking them from the chamber they are exposed to the light of the room, to a lower temperature than that of the bath and to a drier atmosphere. In addition to these changes the leaves suffer a considerable amount of handling. One or more of these factors might have resulted in the observed respiration rise. Direct experiments on the first three of these showed that they could not have caused the observed effect.

Thus if CaCl_2 -dry air is passed over the leaves during an experiment their respiration rate is not affected until they have lost a considerable amount of their fresh weight. Similarly if leaves are exposed to the normal light of the room for 5-10 min. in a glass chamber no change in their respiration can be detected. Independent light experiments have shown that the respiration is put up after exposure, but not to an extent approaching the observed effect unless the illumination is of the order of 48,000 lux for periods of 1-2 hours or more. In the stimulation experiments to be described the leaves were exposed to the very dull diffuse light of the laboratory for periods never exceeding 5 min. Exposure of the leaves to temperatures lower than

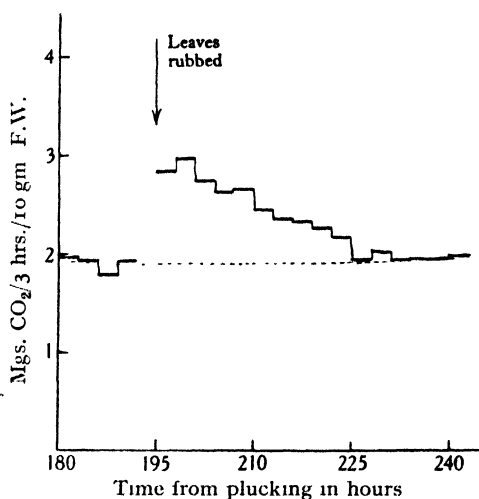


Fig 3 The first effect obtained by deliberate mechanical stimulation of a sample of leaves. This effect was obtained by rubbing both surfaces of each leaf in the sample with a duster.

that of the bath for short intervals also had no after-effect on the respiration. Thus the only alternative cause for the observed effect was the actual handling of the leaves. To test this the leaves were removed from the chamber and deliberately rubbed on both surfaces with a duster. They were then replaced in the chamber and the respiration followed. Fig. 3 shows the results obtained. It will be seen that the effect is identical in form with that of Fig. 2. That it is handling alone that causes the observed effect has been conclusively proved by recent experiments in which leaves are bent by a mechanical arrangement in a chamber which remains in the bath during stimulation. In this way the leaves are "handled" under the same

conditions as they are allowed to respire. The effect of this treatment is the same as that obtained on rubbing the leaves by hand as in the first experiments carried out.

It was found in these preliminary experiments that a relatively small amount of stimulation gave a large effect, and that a more vigorous bending or deformation of the leaf gave no further increase in the respiration. It was decided in subsequent experiments to ensure that the leaves received this maximum stimulation. The following stimulation technique was therefore evolved. The leaves were removed from the chamber and placed on the bench. Each leaf was then taken separately by the petiole between the thumb and forefinger of the left hand. This is shown in Fig. 4. The leaf was then placed on the bench and, with a duster round the forefinger of the right hand, the surface of the leaf was rubbed from petiole to tip. At the same time the petiole end of the leaf was raised from the bench, thus causing the lamina to bend where it was in contact with the rubbing finger. This procedure was carried out twice for both sides of each leaf. The whole procedure is illustrated in Fig. 4. The leaves after this treatment were replaced in the chamber, the chamber sealed and replaced in the bath and the air current through it started. The whole procedure from opening the chamber to restarting the air-current took on the average about 3 min.

THE DRIFT OF THE RESPIRATION DURING STARVATION

Before going on to discuss the stimulation effect on the respiration it will be advisable to give a brief résumé of the normal drift of the respiration of mature starved cherry laurel leaves, since stimulations have been done at varying intervals of time from the plucking of the leaves, *i.e.* after different periods of starvation in the dark. Since the effect varied with its position on the drift it is necessary that the drift itself should be described with a certain amount of detail. This drift was first described many years ago by Blackman (1908), and more recently by Godwin and Bishop (1927).

If leaves of the cherry laurel are detached from the tree and allowed to starve in the dark with their petioles in distilled water, their respiration is found to follow the course illustrated in Figs. 10 and 11. Immediately after plucking the respiration shows a very high value, which varies with the age of the leaf and its position on the tree. It stays at this high value for a length of time varying from 0 to 9 hours and then falls rapidly over a period of about 5 days. After this time it reaches a low value which slowly falls with time.

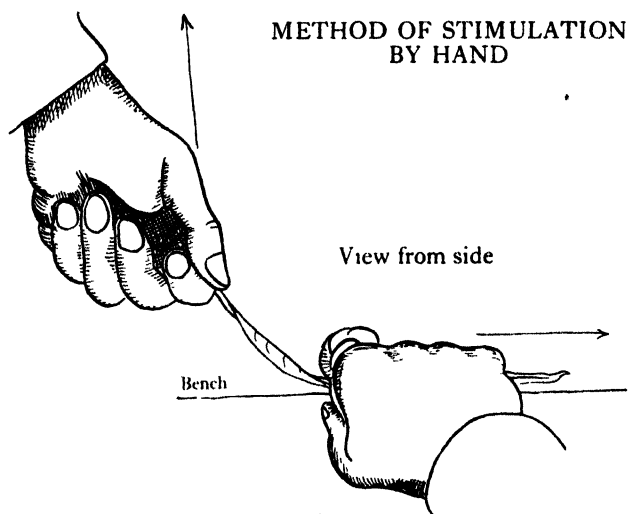
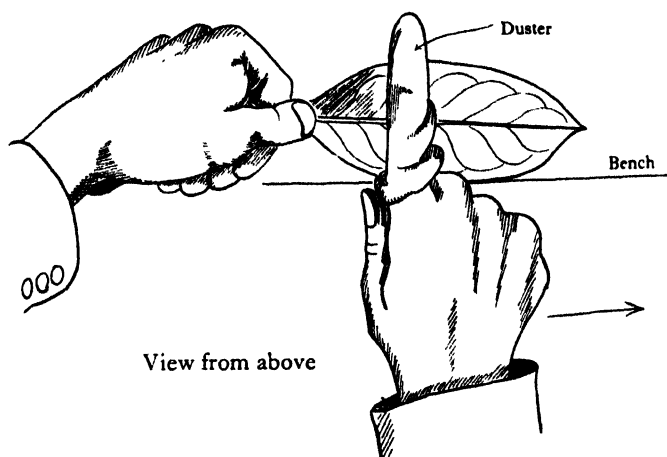


Fig. 4. Drawing showing the final method evolved for the stimulation of leaves. The forefinger of the right hand rubs the lamina, while the right hand by moving upwards bends the leaf at the point of contact with the right forefinger

These initial phases are thought to be true starvation effects, being expressions of starvation changes in the respirable substrate. Thus the initial rapid fall is probably connected with the exhaustion of relatively labile substrates, whereas the slowly falling phase is one of the gradual depletion of relatively non-labile material. It is probable that both these enter in some part into the initial rapidly falling phase.

After a time which varies from leaf to leaf, with age and with time of year, the respiration rises again, and shortly after the commencement of this rise the leaves start to turn yellow. This is illustrated in Figs. 10 and 11. This rise continues for a number of days and then ceases, the respiration turning over into a symmetrical fall. During this rise and fall the leaves show a progressive yellowing commencing along the midrib and gradually spreading thence over the whole lamina. Both this yellowing and the "hump" in the respiration are expressions of the senescence of the leaf and correspond with the breakdown of the protoplasmic organisation of the leaf. The senescent hump in the respiration has been attributed by Blackman to a decrease in the organisation resistance of the protoplasm to the respiratory processes with the advent of senescence. Eventually at some time on the falling part of the senescent hump the differential permeability of the yellow cells of the leaf is lost, and fungi appear on the material which exudes from these cells. The appearance of these fungi is marked by the last rise in the respiration.

THE EFFECT

Having proved that mechanical deformation of the leaves was the sole cause of the observed effect on the respiration, a series of experiments were carried out to determine the form and magnitude of the effect on the respiration of stimulating the leaves at various times during their starvation life. Up to the present a total of five complete experiments have been carried out. In each of these the stimulation effect has been investigated throughout the whole of the starvation life of the leaves, from plucking to the final advent of fungus.

In each series a set of fifteen leaves weighing about 40 gm. were gathered and sealed into the respiration chamber within an hour of plucking. Stimulations were carried out in the manner previously described at intervals of approximately 3 days. In some cases the intervals were longer, in others shorter, the actual time being decided by the duration of the effect. Thus no stimulation was carried out

until the respiration had returned to normal after the previous stimulation, and had remained there for a sufficient length of time for the normal pitch and drift to be accurately determined. Fig. 5 shows the actual observed respiration curves (drifts plus effects) for one of these long experiments. The continuous lines are the actual observed respiration curves. Stimulations were carried out at the times indicated by the downwardly pointing arrows. In this graph the intervals between some of the effects and the following may seem very small. Actually the respiration had returned to normal at least 12–15 hours

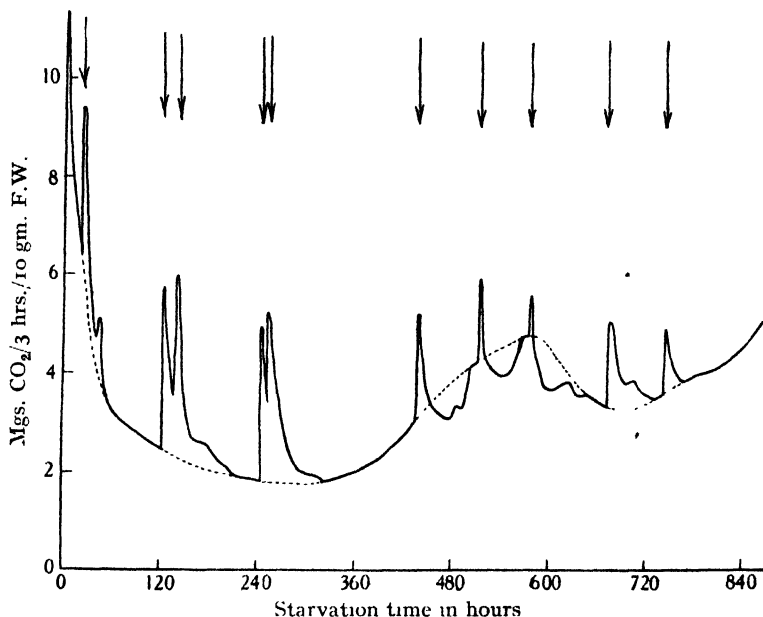


Fig. 5. Smoothed graph of a typical experiment on one leaf sample on a much compressed time axis. The downwardly pointing arrows mark the times of stimulation. The relative magnitude of the normal drift rate and stimulated rate is clearly shown. Note also the depressant effect on the senescent hump.

before the subsequent stimulation was carried out, the great compression of the time scale in these graphs obscuring that fact. Figs. 6 and 7 show two typical effects in greater detail.

The general shape of the effect remains remarkably uniform, with a few exceptions which will be mentioned later, throughout the whole starvation life of the leaf. Immediately after stimulation the respiration shows a very high value which may be as great as three times that before stimulation. It remains, however, at this high value only

for a short time, since the next 3 hours shows a considerable drop in the respiration. This drop continues, the respiration falling away in a smooth curve towards the normal drift, which is reached after varying periods of time from the stimulation. In this typical effect early on in the starvation life of the leaf this time is about 60 hours. In some cases the respiration does not fall along a smooth curve, as in the case of Fig. 6, but shows an interruption in its fall in the shape

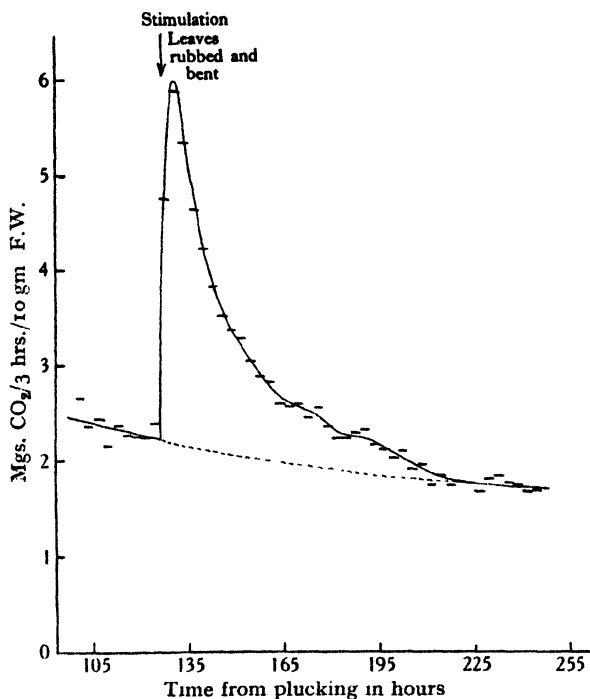


Fig. 6. Typical effect of stimulation giving a smooth return of the respiration to the normal rate. The horizontal lines are the actual respiration readings.

of a step or a secondary rise generally at about 24 hours from the stimulation. This type of effect is seen in Fig. 7. The occurrence of this step is quite sporadic, and, with the data in hand at present, it has not been possible to correlate this occurrence with any particular condition of the leaf or the experiment.

The above type of effect is always observed when stimulation is carried out on the initial rapidly falling phase and the following slowly falling phase of the normal drift. It also holds for stimulations on the senescent hump, but here in a great number of cases, a double

effect of stimulation becomes apparent. This double effect is seen in the previous figure (Fig. 5) and also in Figs. 8 and 9. In these latter graphs the effects have been plotted as differences between the actual respiration and the normal respiration drifts which have been interpolated (dotted lines in Fig. 5). Thus depressions of the respiration below the normal are plotted as negative values. These two curves have been chosen from the results from two different sets of leaves to show that the effect is constant in form when it occurs.

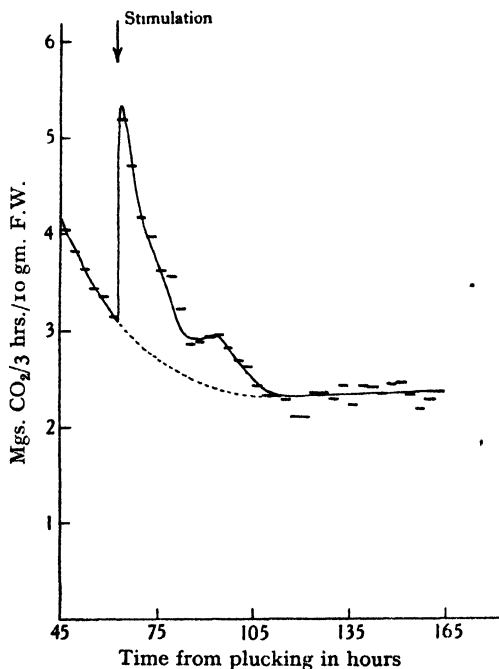


Fig. 7. A second type of effect in which the respiration, instead of falling off smoothly to the normal drift rate, shows a secondary hump after about 15 hours from stimulation.

In this case the respiration shows after stimulation the typical stimulatory rise, but here the fall off is much more rapid, the respiration reaching the normal after about 12 hours from stimulation. Instead of remaining there it continues to fall below normal, showing a minimum value and a slow return to normal. This is the case in the effect of Fig. 8. In the second example there is at first a small dip below the normal followed by a larger dip, the normal being reached after about 50 hours.

Thus here we have two effects of stimulation, the first being the normal stimulatory effect of increasing the respiration, the second a depressant effect on the respiration. The dotted lines in the graph show the probable analysis of the observed respirations into these two effects. In the first case the normal stimulation effect shows a smooth fall off, whereas in the second case we observe the appearance of the secondary hump mentioned previously. One thing is quite clear from these and other similar results and that is that the depressant effect takes longer to develop and acts longer than the normal stimulation

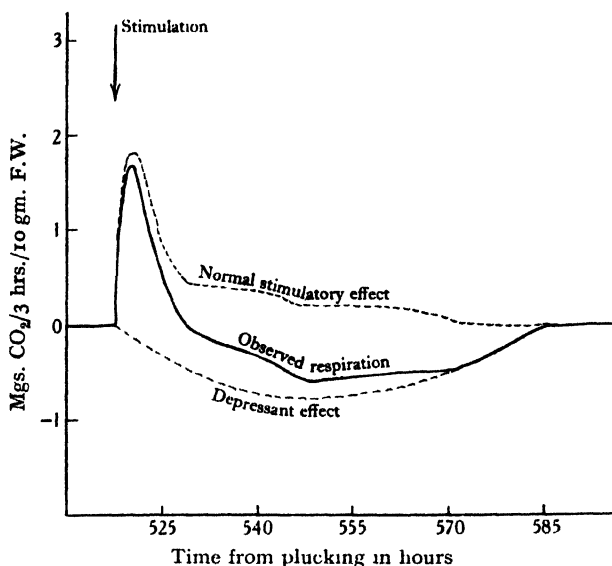


Fig. 8. A third type of effect on the senescent hump. This effect has been analysed into two components, the dotted lines showing their probable drifts with time.

effect. This depressant effect has been noticed only during the senescent hump of the respiration, and it is very probable that its appearance is intimately bound up with the senescence of the leaf. It has occurred in four out of five complete series that have been carried out, but in the fifth it was absent. In the experiment of Fig. 5 and in another similar experiment done at the same time of the year three successive stimulations showed this depressant effect. Its appearance seems therefore to be connected in some way with the time of gathering since experiments done at other times of the year show it slightly or not at all. No correlation with any particular

condition of the leaves showing this depressant effect has been obtained. More data are needed on this point.

The measure of the stimulation effect adopted is the difference between the respiration intensity at the peak immediately after stimulation and the normal drift respiration at the time. This has been called the stimulatory rise. The variations of this stimulatory rise with its position on the starvation drift of respiration, and its correlation with the normal drift will be considered later. The duration of the stimulation effect, where it is not complicated by the

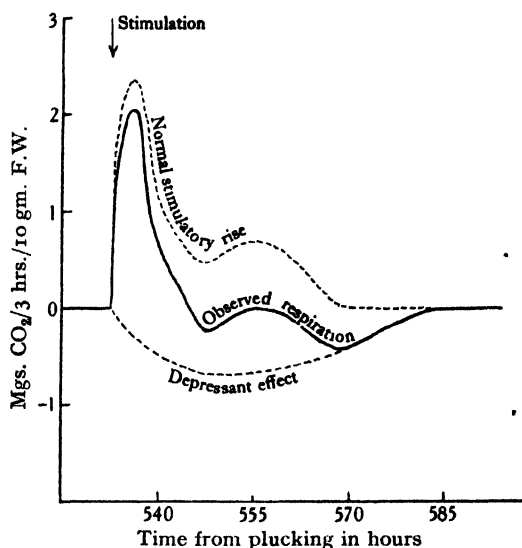


Fig. 9. An effect on the senescent hump showing all three of the possible features of the stimulation effect. Here the normal stimulatory effect shows the secondary hump after about 24 hours. The depressant effect is well marked.

appearance of the depressant effect, shows in general a direct correlation with the height of the rise above normal, *i.e.* with the stimulatory rise. Thus the greater the rise the longer the respiration takes to reach normal after the stimulation. In the case of those stimulations giving rise to the depressant effect, the respiration, as has been previously mentioned reached normal very soon after stimulation and is depressed below it. It is highly probable in these cases that the observed stimulation rise is smaller than the true rise owing to the onset of this depressant effect (see Figs. 8 and 9).

Successive stimulations at short time intervals have been carried out, and it has been found that these successive stimulations will not

increase the respiration to any marked extent above the initial stimulatory rise. This can be seen in Fig. 5. If two stimulations are carried out at an interval of from 3 to 9 hours then the peak of the second stimulatory rise is about 15 per cent. higher than the first, but subsequent stimulations at similar time intervals show peaks in the respiration generally lower than the first. This and similar types of result seem to indicate that there is the development of a fatigue after stimulation resulting in a decreased response to stimulation. This fatigue disappears when the normal respiration is reached after an effect, *i.e.* the full effect is obtained when stimulations are carried out on the normal drift whether there have been previous stimulations or not. In all the series of experiments to be described in which stimulations were carried out at intervals during the starvation life of samples of leaves, the successive stimulations were done in general at intervals of 3 days, thus allowing time for the respiration to reach normal, and thus eliminating any possible effects of fatigue. If the stimulations were carried out at shorter time intervals before the respiration had reached normal, the corresponding effects have not been included in the graphs which follow. In some cases where the effects were small and the respiration very soon reached normal, *e.g.* sometimes on the downward falling part of the senescent hump, stimulations were carried out at shorter time intervals.

The results illustrated in Figs. 10 and 11 are from two typical series of experiments done on sets of fifteen leaves as previously described. In these graphs have been plotted the normal drift of the respiration during starvation, the stimulatory rises and the percentage leaf area remaining green at the time of stimulation on an axis of time of starvation. The percentage leaf area remaining green was estimated by making an accurate full scale drawing of the leaf and sketching the yellow portions by eye. The total area of the leaf and also that of its green portions were planimeted, and the percentage area remaining green calculated from these figures. In Fig. 12 is collected the stimulatory rises for all five experiments. From the above graphs the following facts can be made out:

(1) The stimulatory rises show a falling off as starvation of the leaves proceed.

(2) There is a great variation in this stimulatory rise at comparable times on the drifts in leaves gathered at different times of the year. It must be mentioned here that with the above results, and with results from other, less complete, series of stimulations, there can be observed no correlation between the age of the leaf and the stimulatory

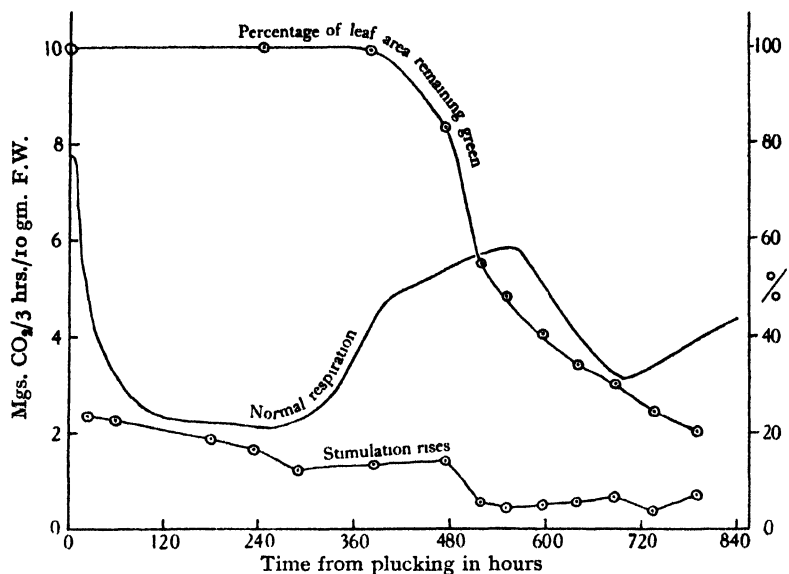


Fig. 10

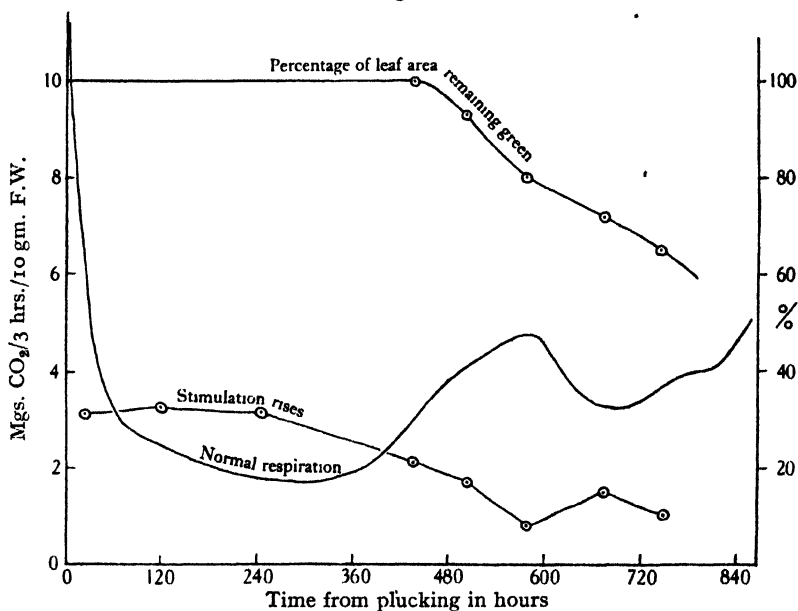


Fig. 11.

Figs. 10 and 11. Graphs showing the relationship between the normal starvation drift of the respiration, the progress of yellowing in the leaves and the size of the stimulatory rise in the respiration. A striking feature is the absence of correlation of the stimulation rises with the pitch of the normal respiration drift. The most rapid fall off of the stimulation rises occurs, however, at the time of most rapid yellowing.

rise exhibited by that leaf. Variations from leaf to leaf obscure any such possible correlation. There is also no apparent correlation between the rise and the pitch of the normal respiration in different leaves at the same period in their starvation life.

(3) There is no correlation between the drift of these rises and the normal respiration drift. Thus the magnitude of the stimulatory rise is independent of the pitch of the respiration at the time of stimulation. This seems to indicate that the stimulation is not acting on the gross respiration, but on some part of the complex which does not follow this gross respiratory drift.

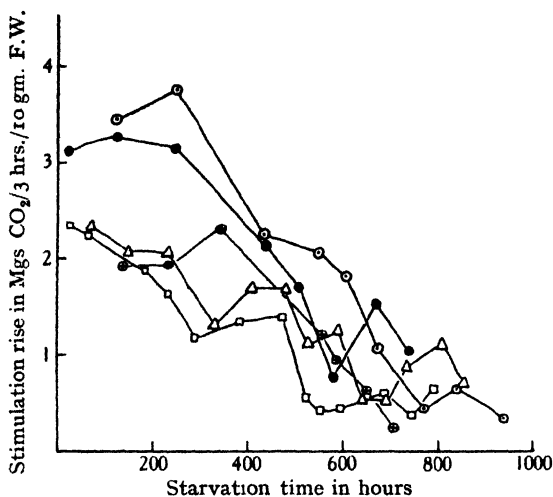


Fig. 12. Graph showing the relationship between the size of the stimulation rise and the starvation time of the leaf sample in hours. Here the tendency for the most rapid fall off of the stimulatory rise to occur at about the time of most rapid yellowing of the leaf can be seen.

(4) The fall off of the stimulatory rises with the starvation of the leaf is not of constant slope, but seems to be more rapid at a time corresponding more or less with the onset of yellowing and the senescent hump. This correlation is much greater in some cases than in others, cp. Fig. 11 with Fig. 10.

These facts show that the effect of mechanical stimulation is independent of the pitch of the normal respiration, and is affected only by the starvation life of the leaf and some intrinsic factor working in the leaf. Thus during senescence the leaf loses its power of being stimulated, and the facts seem to indicate that this loss of power is coincident with the onset of yellowing. The picture that is

formed of the process is that the effect of stimulation shows a gradual falling off during the first two phases of the normal drift. When senescence begins the leaves commence to yellow and to brown, and these yellow and brown cells lose their power to respond to stimulation. Thus during senescence we have a much more rapid falling off of the stimulatory rises due to

- (a) The slow fall noticed in the initial phases, and
- (b) The increase in the number of yellow and brown cells which have lost their power of response.

It would be very instructive if experiments could be carried out until all the cells of the leaf had yellowed. Unfortunately the onset of fungi on the brown parts makes this very difficult. It is impossible to determine directly whether the yellow parts will respond to stimulation, since it is found that in the advanced stages of senescence all the yellowed cells turn brown very quickly and become attacked by fungi before the remaining green cells have yellowed.

The results described in the preceding pages have been of the nature of an exploration into the form and magnitude of the effect as it appears in the normal leaf, observations being carried out on the drift of the effect during starvation, and its variation from leaf to leaf. Having thus acquired a certain amount of knowledge of the "normal" effect it is hoped to carry out investigations under various abnormal conditions, with the view to further elucidating the nature and ultimate cause of this rather striking phenomenon.

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THE EFFECT OF HANDLING ON THE RESPIRATION OF CHERRY LAUREL LEAVES

BY H. GODWIN

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(With 1 figure in the text)

THE reality of the response of respiring plant material to mechanical stimulation demonstrated by Audus in the preceding paper may be confirmed by reference to similar results obtained in 1925 in the course of experiments on the metabolism of starvation in leaves of cherry laurel (*Prunus lauro-cerasus*)(1).

The respiratory drift of detached and darkened leaves was being recorded by carbon dioxide absorption from an air stream passed over the respiring plant material and through Pettenkoffer tubes. A set of leaves had been placed at 20.8° C. in the usual way in a glass leaf chamber darkened with black cloth, and initial readings had been taken when it was decided to transfer the leaves to a metal leaf chamber. This was performed rapidly and without anything unusual in the operation, but the subsequent respiration values departed very strikingly from the course begun already by this set of leaves, and that still followed by the control set which had never been moved. This effect consisted in a sudden rise in carbon dioxide output by about 0.15 of its former rate and a gradual subsidence towards the curve of normal respiration drift such as that shown by the control set (see Fig. 1). This result was so marked that after a time the reverse change to the old leaf chamber was made, and again there followed a definite increase in respiration rate. In each case the leaves had been exposed for 3-5 min. to light, to the dry air of the laboratory and the lower temperature of 14.0° C., and they had been handled. After this various other changes were made for the same set of leaves, and these with their effects can be seen on the graph (Fig. 1). They are also summarised below.

It should be noted that the handling in the first two cases (28 and 95 hours) consisted in no more than holding the leaves between finger and thumb, singly or together, and bending and rubbing them lightly past one another as is inevitable in transferring leaves from

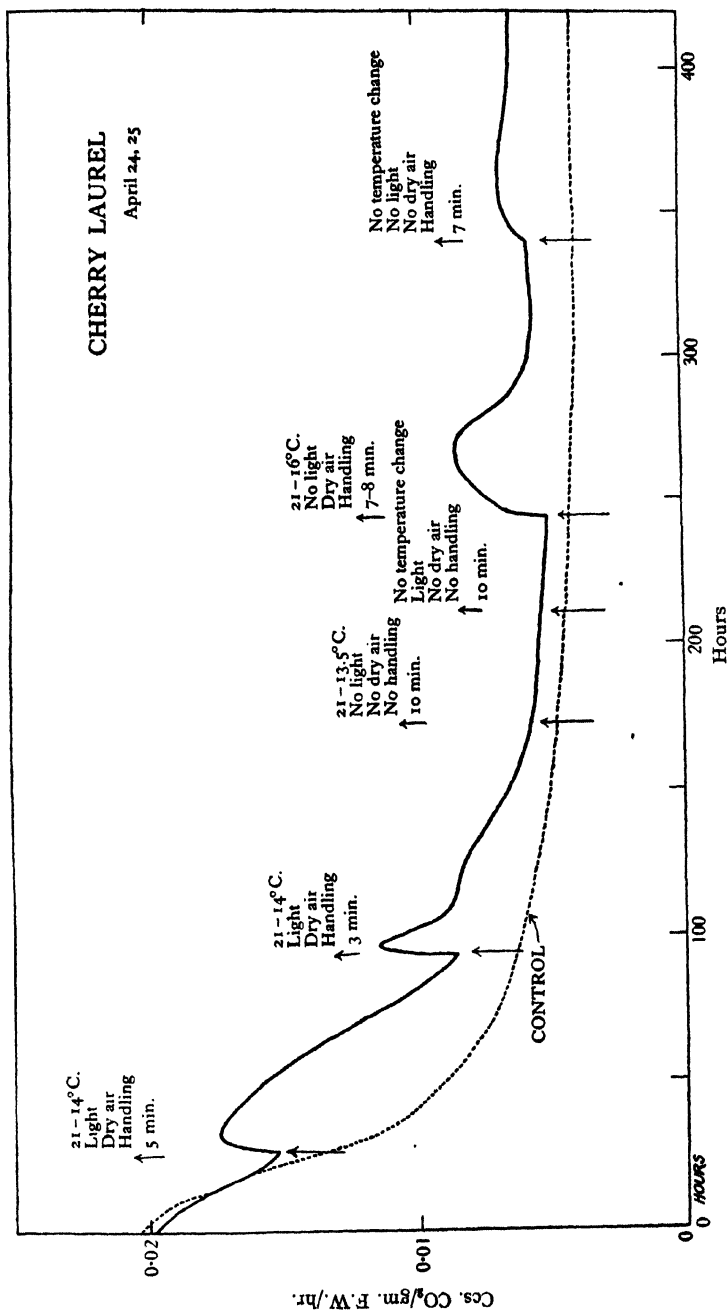


Fig. 1. The respiration rate of two sets of cherry laurel leaves kept at 20-8° C. The control set was undisturbed throughout; the other was at intervals removed for a few minutes from the respiration chamber, and was subjected to the temporary changes of conditions shown by labelling on the graph. Whenever handling has occurred a rise in respiration rate follows.

one small vessel to another. In the two later cases (245 and 341 hours) this was supplemented by light stroking with the fingers.

	Time min.	Temperature change (°C.)	Light change	Humidity change	Handling	Effect on respiration
(1)	5	20.6-14.0	D to light	To dry	Yes	Big increase
(2)	3	20.8-14.0	D to light	To dry	Yes	Big increase
(3)	10	20.8-13.5	None	None	None	None
(4)	10	None	D to light	None	None	None
(5)	7 or 8	20.8-16.0	None	To very dry	Yes	Big increase
(6)	7	None	None	None	Yes	Small in- crease

The results of this experiment point to handling and possibly to exposure to dry air as the causes of the increased respiration, but it is difficult to think that the second factor is indeed operative, in view of the fact that the leaves when taken from the leaf chamber always had moisture upon both surfaces and this was neither wiped off nor dried up. The unimportance of the air humidity change is also suggested by a separate auxiliary experiment performed with a relatively slow gas stream and a single leaf. The air stream entering the leaf chamber was drawn directly from outside the laboratory, and after the normal smooth drift of the respiration curve had been well established the air current was quickened and saturated by passing it through a tower containing wet pumice and kept at the temperature of the water bath. This did not affect the respiration rate, and 19 hours later the tower was replaced by one packed with calcium chloride. Here again for 18 hours there was no change in respiration rate, nor when the original air stream from outside the laboratory was re-established. Throughout the experiment the leaf bases were stood in water and the leaves cannot have been exposed to very severe drying, but at least it must have been as severe as the few minutes' exposure to laboratory air in the first experiment.

The auxiliary experiment was also employed to demonstrate that temperature changes from 20.8 to 13.4° C. and back again, though causing respectively a fall in respiration rate and a recovery to the former drift line, did not cause any after-effect of the type now attributed to handling.

The fact that short exposure to light has by itself no effect on the respiration drift is confirmed by a whole series of experiments in which it was customary to remove the black cover of the leaf chamber every 2 or 3 days, in order to note the progress of colour changes during starvation of the leaf. In this case observation was made through the glass fronts of the leaf chamber and water bath; the

chamber was not opened, and often was not taken from the bath, the process only occupying a few minutes. In no case was there observed any consequent rise of respiration rate above the line of normal drift.

These results appear to show that handling of cherry laurel leaves can cause a large increase in the subsequent respiration rate. If so, it must be a factor of importance in the technique of respiration experiments as well as a factor of great intrinsic interest.

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A NOTE ON THE EFFECT OF HANDLING ON THE RESPIRATION OF POTATOES

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(With 1 figure in the text)

POTATO tubers which possess a firm turgid flesh can be subjected, without serious disturbance of their respiration, to the handling necessary in weighing and placing in a respiration container. Following such treatment, the respiration usually falls slightly initially, but provided the handling has been careful, no further decrease in respiration occurs after 2-3 days in the container. If, however, the potatoes are soft owing to loss of water and senescence, the effect of handling is marked.

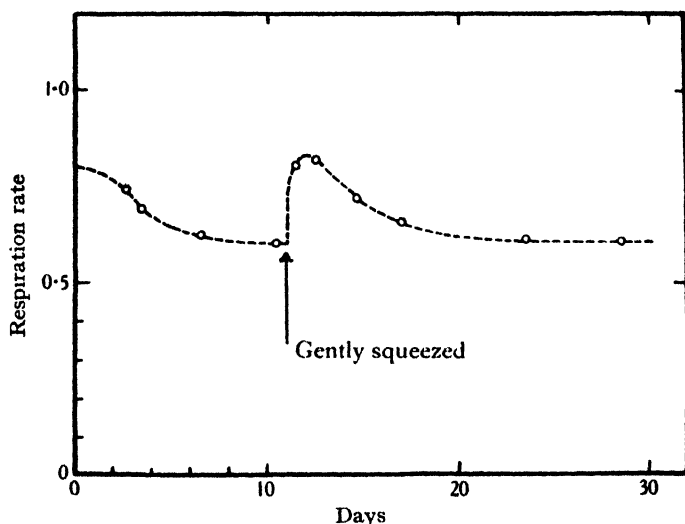


Fig. 1. Effect of handling on respiration of soft old potato.

A soft shrivelled potato, which had been kept at 15° C. for 15 months and had lost roughly 50 per cent. of its original weight, was examined by gentle pressure with the fingers to ascertain the condition of the flesh, weighed and placed in a respiration container.

No measurements of the respiration were made for 2 days, but after this period a fall in respiration of about 20 per cent. was observed (Fig. 1), a level being reached in 8–10 days. After 11 days the potato was removed from the container, gently squeezed and at once replaced. This treatment produced an immediate increase of about 30 per cent. in the respiration (Fig. 1), and the rate did not return to the initial value for some 10 days.

The magnitude of the disturbance of the respiration caused by handling is presumably related to the extent of the compression or deformation of the tissue which occurs. In contrast with firm turgid-fleshed potatoes, the potato used in the above experiment was so soft that even slight squeezing caused a visible compression. On the other hand, the respiration of such soft potatoes was but little disturbed if compression during handling was avoided.

A GENETICAL INTERPRETATION OF THE ORIGIN OF HETEROSPORY AND RELATED CONDITIONS

BY H. C. PINCHER

BROADLY outlined, the generally accepted interpretation of the origin of heterospory from a homosporous condition is as follows: The two types of spore were derived from one which initially resembled the microspore. The megaspores arose in certain sporangia by reduction in the total number of the contained spores and the consequent increase in size of the remaining ones, due to the increased nutrition brought about by this abortion. This change in size of the megaspores was brought about gradually, since series could be traced from a many-spored megasporangium to one containing a single tetrad. Thus Williamson and Scott (1894) showed the presence of abortive nourishing spores in the homosporous *Calamostachys Binneyana*, whilst *C. casheana*, a definitely heterosporous form was considered as a stage further on. Later (1906) Thoday showed that in *Sphenophyllum Dawsoni* the size of the spores varied with the amount of the abortion.

Shattuck (1910) obtained experimental results which he interpreted as illustrating that *Marsilia quadrifolia* would, in culture, repeat all the phases in the development of heterospory reported for *Calamostachys*. It appears, therefore, that the first differentiation of the spores was one of size, the larger always producing the female gametophyte.

A general study of cytogenetics leads to the view that *all* the characters possessed by an organism are in the first place determined by its genes. This view, however, is not universally accepted, since there is a school of thought which, although admitting the genic control of vegetative characters, holds that the phenomena of sexuality have no connection with hereditary units.

This conception has been vigorously supported by Schaffner (1921*a*, 1922*b*, 1929, 1930), who regards sex in plants as a purely physiological condition and suggests that since this condition has no relation to sex factors, cells may be non-sexual or potentially sexual. He therefore distinguishes three types of tissues and organs, viz. neutral, female

and male, and on this ground states that sexuality cannot be determined by genes since such a supposition would demand neutral genes for neutral tissue. In this last statement he is overlooking the power of latency of the gene, an incontrovertible fact which he previously accepted when discussing the awn character of *Zizania aquatica* (1918). In this plant Schaffner showed that the lemmas of the staminate spikelets are awnless, whilst those of the carpellate spikelets have long awns. He admitted that the character of the awn is due to the activity or latency of the awn factors. This latency he attributed to a physiological condition determining maleness but having no connection with genes. The presence or absence of the awn is a secondary sex character, and if we admit this to be controlled by a gene, then we must consider other sex characters to be similarly controlled.

The assumed ancestral homosporous sporangia contained spores which normally gave rise to a gametophyte bearing both male and female reproductive organs, therefore the genes which ultimately determined maleness and femaleness of the reproductive organs must both have been present in the homospore.

Thus the development of the megaspore from the primitive homospore within the sporangium was more than a mere increase in size, since it also necessitated some suppression of the action of the genes determining maleness. Similarly, in the development of the microspores the action of the female-determining factors must have been restrained.

We are thus forced to the conclusion that two separate and simultaneous sex suppressions took place in two separate sporangia. That two such mutations should have occurred at the same time and in the same direction is peculiar, and this peculiarity is intensified when we realise that the same process must have taken place in different groups of plants in the same manner. Moreover, as it was held that the change was gradual, a large number of mutations must have occurred in each case.

Work in recent years has demonstrated the fact that most existing and late fossil groups are geologically much older than formerly supposed, and the derivation of such groups from a primitive type as visualised by Lignier, appears even more probable.

Such a type, with cauloids which became modified into megaphylls, and phylloids which became microphylls, is apparently provided by *Psilophyton*, and even if this is not so we do know that the Psilophytales constitute the oldest known undoubted land plants, and therefore it is in that group that we should look for primitive

sporing conditions. That the Psilophytales were homosporous with regard to size is certain, and that the spores were homosporous with regard to genetic constitution is probable. Thus we may assume that the spores gave rise to gametophytes bearing male and female organs, since the grouping of the spores into tetrads, indicating a meiosis, presupposes a syngamy. We can therefore be fairly certain that in the nature of their gametophytes, the Psilophytales resembled the homosporous ferns. In these forms the spore must contain genes which determine both maleness and femaleness, but as the spore is already haploid the segregation of the two sexes in the gametophyte cannot be of the nature of a chromosomal segregation.

As long ago as 1887, Buchtien (cited by Shattuck, 1910) noted the fact that in *Equisetum* where the male and female prothalli are separate, but the spores homosporous as regards size, the sex of the prothallus is largely controlled by environment. This observation has since been confirmed by other workers, Shattuck (1910) showing that sex is determined long after germination of the spore, when the prothallus consists of many cells. Only one interpretation of these results is possible; namely that the spores are all similar in the fact that they contain factors for both maleness and femaleness, and that under certain influences the action of one or the other of these sets of factors is suppressed, a unisexual condition resulting.

Experimental animal embryology has shown that the inherited genes alone are insufficient to account for the development of an animal. The genes merely enable the embryo to react in definite ways to various influences external to them. If these influences are abnormal, development may also be abnormal. As a result of experiments with *Lymantria*, Goldschmidt (1934) has shown that the structure of the adult as to whether it is male, female, or intersex, depends in part on the relative speeds at which two opposing sets of genes produce their effect. The condition in *Equisetum* appears to be parallel. In the gametophyte, the two opposing sets of sex-determining genes are present in every cell, and under certain conditions the rate of reaction of one set of genes is more rapid and begins earlier than that of the opposing set, a definite sex thus resulting. According to this reasoning, all the spores from a sporangium under exactly the same conditions should give rise to gametophytes of the same sex. This result has actually been realised by Shattuck, who obtained cultures from sporangia which were practically all males.

That the state of affairs is one of suppression of gene reaction and not a destruction of genes is supported by the fact that antheridia

may occasionally develop on female prothalli and archegonia on male gametophytes. Moreover, even at antheridium and archegonium formation, no segregation or absolute suppression has occurred, since filamentous structures regenerated from the basal cells of the sex organs themselves can be made to exhibit characters of both sexes (Czaja, 1921, cited by Sharp, 1925). These abnormalities are easily explained as due to local conditions allowing of rapid reaction of the set of genes determining the development of the organs of the opposite sex. Shattuck (1910) described some experiments with *Marsilia quadrifolia*, a heterosporous form in which he treated the sporocarps, so as to cause abortion of the megaspores in their early stages, and then placed them under favourable conditions. Sometimes only a few of the microspores survived the change, but the surviving ones would respond vigorously, becoming as much as sixteen times the normal size and developing a thick wall and other megaspore characters. He interpreted his results as related to nutrition, and suggested that heterospory in *Marsilia* was attained gradually. These experimental results and many others are in direct line with Goldschmidt's work, and strongly support the statements made above, as will be shown later.

Thus the question of sex in the forms so far mentioned appears to depend on differential rate of gene reaction, but obviously a very important factor is the state of development of the plant, when these sex-determining genes begin to have an effect.

A consideration of sex-chromosomes is desirable at this stage. The most primitive mechanism appears to be the XX - XY type. Let us consider a case where the male is the heterozygous sex. Either of the X -chromosomes of the female can produce a male in combination with the Y -chromosome: therefore these two X -chromosomes are identical and homologous. Either of the X -chromosomes of the female can produce a female in combination with the X -chromosome of the male. In the newly produced female, these two X -chromosomes will act similarly to those of the mother, and thus it is obvious that all three X -chromosomes are identical and homologous. Cytological evidence indicates an homology between the X and the Y , and thus we can be fairly certain that primitively this mechanism consisted of two homologous pairs, one chromosome of which became changed to form the Y .

In a typical XX - XO type, the XX combination results in a female, and the XO in a male. There are only two practicable explanations of this phenomenon:

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(1) The X -chromosome of the male bears male genes, and since all the X -chromosomes must remain identical, then all the X -chromosomes must bear male genes. Similarly they must all bear female genes. Hence sex is determined by the suppression in the zygote of the action of one or the other of these sets of genes.

(2) The X -chromosomes all bear female genes and the male-determining genes are situated on the autosomes. This explanation is also applicable to XX - XY types if we consider the Y -chromosome to be functionless in sex determination. Now this last condition has been demonstrated for *Drosophila* by Bridges (1925) where, although a Y -chromosome is present, maleness is determined solely by the autosomes. Moreover, its wide application is strengthened by the fact that in plants as well as in animals a series can be traced from an XX - XY mechanism, through types showing gradual elimination of the Y -chromosome to an XX - XO mechanism, seemingly without any change in the working of the sex mechanism.

Whichever of the two schemes we take as being representative, we are faced with the fact that the sex of the zygote is determined by suppression of the action of the genes of one sex or the other. Adopting the latter interpretation as more likely, the primitive condition would be a case where all the zygotes had the formula $2A + 2X$, where A represents the autosomes. Such a condition probably obtains in *Equisetum*. Reduction division takes place at sporogenesis, but as would be expected there is no segregation of sex, since each spore has the formula $A + X$.

A type of sex determination such as exemplified by *Sphaerocarpus Donnellii* (Allen, 1919) can easily be derived from the *Equisetum* type. If instead of the initiation of the sex-gene reaction taking place in the partially developed prothallus, it be coincident in time with meiosis, and if in conjunction with this a differentiation occurs between the X -chromosomes, then an X - Y mechanism will obtain. The male and female spores arise automatically. The spores of *Sphaerocarpus Donnellii* are homosporous with respect to size, but since their sex is determined from the beginning they are to all intents and purposes heterosporous. This condition, rather than a size differentiation, is held by the writer to represent incipient heterospory.

We have seen that sex determination can arise concurrently with meiosis, or a long time after it. Obviously it can arise before meiosis just as simply! Suppose the competition between the two sex-determining sets of genes begins at sporangium formation. In some of the sporangium primordia the female genes obtain supremacy and

a sporangium develops giving rise to female spores alone. By a similar process microsporangia arise. Such a condition is to be found in *Selaginella*. It is significant that such a variable factor as the position of the strobilus conditions the development of megasporangia. Thus in dorsiventral strobili they are ventral in position, whilst in pendent strobili they may occupy the apex. An important point in connection with this idea of the shifting of the time of sex determination lies in the fact that we can expect an immediate difference, not only between the two kinds of spore, but also between the two kinds of sporangia. One of the consequences arising from this change appears to have been the increase in size of the female spore mother cell, and in connection with this a necessary competition for the limited food supply arose, only a certain number of spore mother cells being successful. This competition has been experimentally demonstrated by Shattuck in *Marsilia quadrifolia*. Since this reduction in number is due to active competition, we should expect some cases where two or more spore mother cells tie in the race for food, a larger number of functional megaspores arising than is normal. This condition has been shown not only in *Selaginella* (Duerden, 1929), but also in *Bothrodendron mundum* (Holden, 1932). Moreover, as we should expect, these spores are smaller than the normal ones in both cases.

Thus the presence of abortive spores was a consequence of the increase in size of the megaspores and not the cause of it, as the older theory demanded. In this light, Thoday's observation that in *Sphenophyllum Dawsoni* the size of the spore varied with the amount of the abortion is easily explicable, since the larger the spores were initially the fewer would be successful and more would consequently atrophy. The use of these abortive spores as food supply for the successful megaspores was incidental to their formation. Realising that sexual differentiation can occur in the diplophase, it is but another step to the establishment of a monoecious condition. Thus, for example, in a certain region of a plant, the environmental conditions have increased the rate of reaction of the genes of one sex and branches arise bearing the reproductive organs of that sex only, but since the genes of the other sex are still latent there and possess the power to act under the right conditions, sex mosaics and intermediates are easily explicable.

A dioecious condition is attained by the establishment of sex immediately after syngamy. The different degrees of monoeciousness expressed in different plants supports this contention. Schaffner, however (1918), holds that although in some cases the monoecious

condition may be a step in the direction of dioecism, this latter usually arises through a succession of more extreme vestiges; that is in different plants different sexes become vestigial. This improbability need be held no longer, and in the light of the facts presented above, it will be seen that these "vestiges" are rather to be regarded as rudiments. A differential sex-chromosome mechanism is not necessary for the establishment of a dioecious condition, and consequently sex reversals under abnormal conditions are to be expected. Sex reversals and intermediates are well known and have formed the greater part of the evidence against genic sex determination.

From such a dioecious condition or directly from a monoecious condition, an XX - XY dioecious type can be derived by loss of function of one X -chromosome in the male. The function of such a mechanism might be questioned, since Schaffner has shown that sex reversal can be brought about in *Cannabis sativa* (1918, 1921*b*, 1929) and *Humulus japonica* (1923), both of which possess an XX - XY -chromosome set. The determination of sex in these forms is certainly not so fixed as in animals, but it would appear that it is somewhat better delimited than in cases without the mechanism. Thus in *Mercurialis annua* (Yampolsky, 1919, 1920*b*) periodic sex reversals occur as a consequence of normal seasonal changes, whilst *Cannabis sativa* (Schaffner, 1918, 1929) is strictly staminate or pistillate under normal conditions, only showing sex reversal when subject to abnormal environment.

The evidence so far presented warrants the generalisation that the sexual condition of a plant depends on two factors: (*a*) the particular stage of ontogeny which has been reached when sex establishment begins; (*b*) the reaction of the sex-determining genes to conditions external to them. The most important of the external conditions appears to be nutrition. This conclusion was reached by Shattuck in his experiments with *Marsilia quadrifolia*, which have been described earlier in this paper. His results may now be interpreted as follows:

Sex determination has occurred in the diplophase between sporocarp and sporangium formation. The spores contain both male and female genes, one set of which is dominant in each case. Up to a certain point in the development of the microspores, the male-producing genes have gained the upper hand, since the normal external conditions have favoured their reaction. Under the unfavourable experimental conditions, the megaspores and many of the microspores abort and cannot be revived. However, when favourable

conditions are re-established the female genes are favoured and gain the upper hand, female structures thus arising.

There is no dearth of experimental evidence in favour of the fact that in plants sex expression is controlled by environmental factors. Nagai (1915) showed that the development of sex organs in homosporous fern prothallia could be controlled by varying cultural methods. Kraus and Kraybill (1918, cited by Talley, 1934) have demonstrated that the relationships between carbohydrates and nitrogenous materials are determining factors in the development of sex organs in several bisporangiate angiosperms. Camp (1929) examined twelve species of plants and found that in every case the tissues related to the male structures showed a greater catalase activity than the tissues related to the female. It was also found that the floral structures showed a greater catalase activity than the vegetative parts.

Talley (1934) has shown that it is probable that in *Cleome spinosa*, the staminate flowers arise from regions lower in certain nitrogenous elements than the regions giving rise to the pistillate flowers, and that possibly a similar state of affairs exists in *Cannabis sativa*. Sex reversals in plants have long been known to occur as a result of environmental influence. Such reversals have been brought about by cold (Shattuck, 1910), mutilation (Higgins, 1916; Pritchard, 1916), by varying light intensity (Shattuck, 1910; Schaffner, 1918, 1929), and by varying moisture conditions (Schaffner, 1922 b).

Sex reversals as a result of parasitism are well known, the classical examples being those of *Lychnis dioica* when attacked by *Ustilago violacea*, and *Clematis foetida* when attacked by *Aecidium ottagense*. From such diverse results, it appears probable that all dioecious sporophytes would exhibit sex reversal under certain conditions.

The particular stage of development which has been reached when sex establishment begins is fairly constant for a particular species, so that it appears certain that this character is controlled by a gene or genes. This being the case, it will be seen that by a single mutation in this gene or gene set, the whole sexual condition of the plant can be altered. Thus, for example, a heterosporous condition of the *Selaginella* type can at one stroke be derived from the homosporous condition of the related *Lycopodium*, and such a gene mutation would of course be permanent. It is typical of *Selaginella* that only one tetrad of megaspores matures, and at first sight it might appear that the above suggestion disposed of any relationship with the fossil *Selaginellites*, where a larger number of megaspores per megasporangium was the

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rule. It will be understood, however, that vegetative mutations will affect the environment of the sex genes to some extent, and consequently the development of the reproductive organs also. Therefore some difference in this respect is to be expected and is shown in the fact that in *Selaginella* a smaller number of megaspores mature. The actual time of initiation of the sex-gene reaction is the same in both genera.

In this way, many anomalies within different groups of plants can be accounted for, the most obvious probably being the sporadic occurrence of heterospory. Another is afforded by the relationships of the Marsiliaceae and the Salviniaceae. Formerly these families were placed together in a separate group, the Hydropterideae, but Bower has shown that the Marsiliaceae are more nearly related to the Schizacaceae than to the Salviniaceae. The apparent relationships of the two aquatic families are easily accounted for as a result of parallel mutations in two widely different plants, causing the onset of the sex-gene reaction at about the same stage in the life history. It is significant that the mutations were slightly different in that in the Marsiliaceae where the male and female sporangia are borne in a single sporocarp, sex determination takes place between sporocarp and sporangium formation, whereas in the Salviniaceae, where the sporangia are in separate sporocarps, it occurs before or at sporocarp formation. That it was probably the latter is suggested by the fact that in the very early stages the sex of the sporocarp primordium cannot be distinguished. This phenomenon of parallel mutations is of widespread occurrence and has been known for a long time, being first recorded in *Oenothera* (Gates, 1912). Another instance of its application is afforded by the hypothesis put forward by Boyd Thomson (1927, 1934) which regards the seed plants as derived from a primitive *homosporous* Eusporangiate stock. The evidence for this suggestion is considerable, and as will be readily seen, the idea is consistent with the views expressed above. He points out that in heterospory there is usually a great difference in spore size and little difference in the size of the sporangium, whilst in the seed habit there is little difference in spore size but great difference both in size and elaboration of the sporangium. This seed habit he therefore terms "heterangy" in contradistinction to heterospory. It will be seen that heterangy is very similar to heterospory in that it can be derived by a single mutation from a homosporous condition, the mutation being such that a differentiation in sporangium size obtains, rather than a differentiation in spore size. The important point to realise is that

heterospory and heterangy are independent mutations, and the latter is not brought about by modification of the former.

Thomson also points out that in all heterosporous forms the microspore develops its prothallus endosporally as also does the megaspore, and he regards this as indicating that intrasporal prothallial development preceded heterospory, and was characteristic of the parent homosporous stock. Thomson himself, however, and later Gates (1932), have shown that size is no criterion of heterospory, since in some cases, the microspores are larger than the megaspores, and it has been pointed out above that genetical heterospory is the fundamental distinction. *Sphaerocarpus Donnellii* is therefore a fundamentally heterosporous type in which the prothalli are *not* endosporal.

A mutation from the *Equisetum* condition to a condition such as exemplified by *Selaginella* will bring about a sudden change of external influences as far as the sex-determining genes are concerned, since these latter will begin their reaction in contact with absolutely different tissue. These influences will greatly affect the development of the gametophyte as long as it remains within these conditions. Thus the production of internal reduced gametophytes is explicable, and there is no necessity to assume an endosporal prothallial development in the immediate ancestry.

In types with male and female sporangia, the sex-gene reaction commences at or before sporangium formation, and so at the time of the shedding of the spores the genic reaction will be in progress. Under favourable conditions, this reaction will continue and the development of the gametophytes will be rapid. In homosporous forms, however, this reaction begins late in the development of the gametophyte. Thus are the words of Campbell (1918) explained: "In strong contrast to the slow growth and late development of the reproductive organs in homosporous forms, most heterosporous Pteridophytes germinate very quickly." In connection with this, the fact that the sex characters of the gametophytes of *Sphaerocarpus* develop very early is a significant one.

Of recent years there has been a reaction against the unlimited application of peculiarities of the reproductive organs in the determination of plant phylogenies, and in the light of the facts presented above it will be clear that this reaction is in the right direction, since parallel mutations in two widely separated plants may give rise to two plants identical in certain features of their reproductive organs. This is what appears to have happened in the case of *Marsilia* and *Azolla*.

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More attention has of late been paid to vegetative characters, particularly those of the vascular system, in attempts to discover relationships. This attitude has met with a good measure of success, and a realisation of the views suggested here should render further investigations in the field of physiological anatomy even more fruitful.

In conclusion I should like to express my gratitude to Prof. R. Ruggles Gates for his valuable criticism and unfailing help and advice.

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THE DEVELOPMENT OF THE SPIKELET IN *AGROSTIS CANINA* L.

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(With 5 figures in the text)

INTRODUCTION

THE earlier stages in the development of grass spikelets have rarely been investigated with any precision, and most of the accounts which have been published are concerned purely with the external morphology of the immature spikelets. Some very careful work of this kind has been carried out from time to time in the last century, but no comparative study within the family has been attempted. Excellent figures are given by the following authors: Wigand (1854), Payer (1857), Goebel (1884, 1895), and Schuster (1910). The anatomy of the young spikelets has been studied less, and the authors who have written on this subject have been concerned chiefly with the formation of the sexual cells (Cannon, 1900; Weatherwax, 1917).

In the present paper an account is given of the development of the spikelet in *Agrostis canina*, as seen both externally and in serial sections. The spikelet of the genus *Agrostis*, with its single flower, is suitable for such a study, as it is reduced to its simplest terms while still retaining the characters of a typical grass spikelet. *A. canina* was chosen in particular because of the presence of the dorsal awn. The development of this structure is of some theoretical importance, as it has been usual to regard it as homologous with the blade of the foliage leaf. After the description of the development of the spikelet in *A. canina* the morphology of the dorsal awn will be briefly discussed.

The development of the spikelet is inseparable from that of the inflorescence as a whole, and figures of the external morphology of the young inflorescence are given by most of the authors who figure the young spikelets. A short account of the development of the inflorescence is given in the present paper as an introduction to the account of the development of the spikelet.

The sheath of the uppermost leaf of the flowering culm serves as

a protection to the developing inflorescence. The axis above the node of this leaf remains very short, and develops a regular system of lateral branches in alternate semi-verticils. The branches of the upper verticils, which are formed last, remain small even in the mature panicle, forming a terminal cluster of branches. The lower and earlier formed verticils have extensive branch systems which subdivide repeatedly forming a compact mass which overlaps the verticil above. The first formed verticils lag in their development behind those formed later, so that, at the early stage shown in Fig. 1, the largest verticils are at about the middle of the panicle. Those verticils above

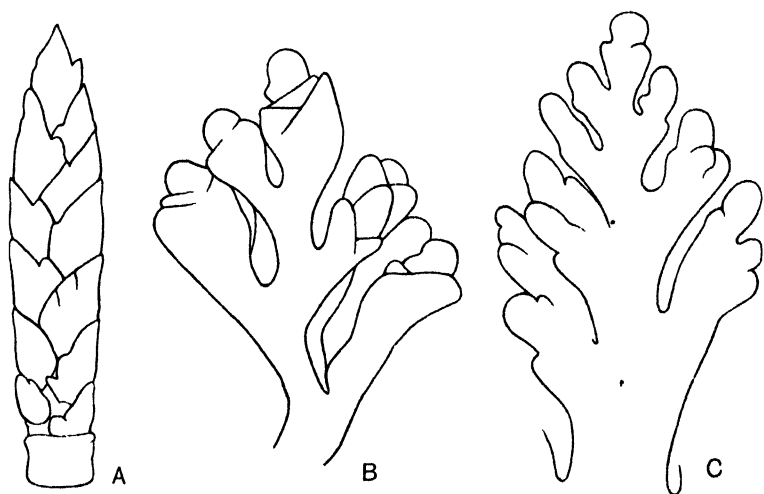


Fig. 1. Young inflorescence of *A. canina*. A, complete inflorescence, $\times 12$; B, uppermost branches, $\times 125$; C, main branch of lowest verticil, $\times 125$.

are smaller now as in the mature panicle, and those below, although formed earlier and finally larger in the mature panicle, are now smaller, developing at a slower rate the lower they are on the rachis. Trécul (1880) records exactly the same method of development in the panicle of *Phleum asperum*: "Il arrive que, de très bonne heure, le deuxième rameau, puis le troisième, le quatrième, etc., croissent plus vite que celui ou ceux qui les ont précédés, en sorte que bientôt ils l'emportent tellement sur ceux-ci, que, sans un examen attentif, on pourrait les croire nés avant eux." Helm (1934) figures inflorescences showing this type of development for the species *Monerma repens* (Fig. 2) and *Stenotaphrum subulatum* (Fig. 4).

The panicle is very minute when all the verticils have been formed

and enlarges very gradually as the lateral branch systems develop. When the panicle is about 4 mm. long (Fig. 1 A) further elaboration is stopped by the tips of the uppermost branches beginning to become differentiated as spikelets by the formation of ridges which will develop into the glumes (Fig. 1 B). Although the branches are formed from below upwards, it is the upper branches which first bear rudiments of the spikelets. The branches of the lower verticils at the same stage (Fig. 1 C) are still actively dividing, forming the intricate branch system of the mature panicle. The upper spikelets are the first to leave the protective sheath, and they retain their advanced condition until the time of flowering. The rudiments of the glumes appear at the ends of the branches of the verticils in a downward succession, and by the time the lowest branches show signs of developing spikelets the panicle has only elongated by about another millimetre.

The culm between the base of the panicle and the uppermost leaf remains very minute until an advanced stage in the development of the panicle. As the spikelets become more highly developed the branches which bear them elongate, together with the rhachis, until the whole of the protecting leaf-sheath is filled and the upper and more advanced spikelets protrude. The lowest verticils are still only slightly differentiated, but as the top of the panicle slowly protrudes their development is completed. At this stage the culm has attained the length of about a centimetre or a little more, that is it is about the same length or shorter than the first internode of the rhachis. Now the culm elongates rapidly to eight or ten times its previous length, pushing the whole panicle free of the protective sheath. The branches which have remained in a close bundle now spread out to form a diffuse panicle and the flowers open in a series from above downwards, anthesis usually lasting three or four days. In this and most other European species of *Agrostis* the branches become raised against the rhachis again after flowering. The upper branches may be already slightly contracted when the lower spikelets are still in flower; the lower branches are raised more slowly and the degree to which they are contracted varies in individual plants.

EXTERNAL MORPHOLOGY

As has already been described the development of an individual spikelet is initiated by the appearance of the crescentic rudiments of the glumes below the apex of the branch, which thus becomes the rhachilla. The differentiation of the spikelets takes place in a series

from above downwards, not only in the panicle as a whole but in the individual branches of a verticil. In Fig. 1A a panicle is shown at the stage when all the verticils have been laid down and spikelet formation has just begun. In Fig. 1B the extreme apex of the panicle is shown more highly magnified, the terminal flower, and those of the lateral branches also, show rudiments of the glumes and, in some cases, of the lemma also. The whole of the main branch of the lowest verticil is shown in Fig. 1C; the branches are still dividing to give

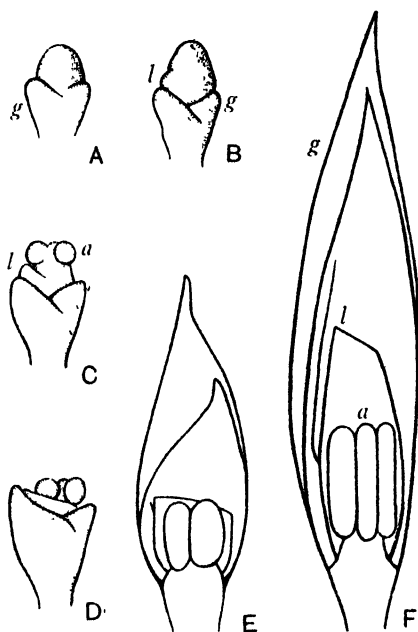


Fig. 2. Successive stages in the development of the spikelet. A-D, drawn under the binocular microscope, $\times 125$; E, F, diagrammatic, $\times 60$. *g*, glumes; *l*, lemma; *a*, anthers.

rise to the diffuse panicle, and their tips show no indication of the glumes.

It is possible to make out much of the development of the spikelets from dissections under the binocular microscope. A series of spikelets at successive stages of their development is shown in Fig. 2. The horns of the crescent-shaped rudiment of the first glume extend around the pedicel, but the margins do not overlap as do those of the foliage leaf. The second glume appears almost simultaneously with and very slightly above the first (Fig. 2A), but the

lower glume develops more rapidly at first, and soon encloses the whole of the young spikelet. Before this occurs, however, the floral axis can be seen to give rise first to a lemma rudiment (Fig. 2 B) and then to three stamen rudiments (Fig. 2 C), leaving the central apex to become transformed later into the ovary.

The various parts of the spikelet not only appear in order from below upwards, but continue to mature in that order. As has been said the outer glume soon encloses the whole spikelet and it continues to develop until it soon reaches its full length. The second glume does not lag far behind, so that by the time the glumes have attained to nearly their full size the other organs are still comparatively rudimentary. The lemma appears very early (Fig. 2 B), but develops slowly and does not enclose the anther rudiments until these are well developed with a connective and four lobes. When it is about the length of the anthers and wraps them about completely, the rudiment of the awn appears on the back near the apex (Fig. 2 E). The whole of the lemma is still meristematic and now elongates rapidly, soon becoming twice or more the length of the anthers. The awn develops slowly at first remaining shorter than the lemma, but when the lemma has nearly reached its full size the awn lengthens rapidly and in a typical spikelet of *Agrostis canina* soon projects beyond the glumes. The growth of the lemma must be chiefly apical, as the distance between the base of the lemma and the insertion of the awn increases only slightly (Fig. 2 E, F), whereas the insertion of the awn changes during development from subapical to below the middle of the lemma. The palea is very small in *A. canina*, and its development and that of the lodicules can only be followed with any certainty in serial sections. The stamens form very early as three spherical swellings below the apex of the flowering axis. They become elongated and furrowed with the formation of the anther lobes. Until they have become about one-third their mature length, the anthers are sessile on the floral axis, below the ovary, but when the lemma has nearly completed its growth the filaments begin to elongate, pushing the anthers towards the apex of the lemma. The anthers increase in length at the same time, being about two-thirds the length of the lemma at maturity. At the time of flowering the filaments elongate further and the stamens protrude freely from the gaping glumes and lemma. The early stages of the formation of the ovary are best seen in serial section. By the time the stamen filaments are ready to begin elongating, the ovary is a cylindrical structure, with two short, simple, rod-like style rudiments. Very little further change

takes place before fertilisation; the ovary becomes more elliptical with the development of the ovule, and the styles elongate and develop a much-branched stigmatic surface.

SERIAL SECTIONS

Parts of young inflorescences including spikelets in several stages of development were fixed and embedded in paraffin wax and series of microtome sections cut in transverse and longitudinal section. By a study of these series it is possible to get a more precise knowledge of the development of the parts of the spikelets and to understand their relationship to one another.

The glumes

The crescentic rudiments of the two glumes appear almost simultaneously, the later to form being slightly higher on the pedicel. The horns of the crescents extend around the axis as the rudiments elongate; they do not completely encircle the axis and fuse together as do those of the vegetative leaves, but stop when separated by about one-quarter of the circumference. Their bases fuse to the axis obliquely so that the middle of the lower glume is attached to the axis in sections in which the margins of the upper glume are free.

While the upper glume is still meristematic its upper part grows very quickly in length, becoming narrow and acuminate, but the base grows in breadth and encloses the other parts of the spikelet. The upper glume is enclosed except at the back of the mid-rib, and soon becomes almost equal in length to the lower. The epidermal cells of the keel and sometimes those of the margin, near the apex, develop into teeth in a downward succession.

The bases of the glumes are cushion-like, with several layers of mesophyll between the two epidermal layers, but in their upper parts the glumes consist of only the large-celled inner and the small-celled outer epidermis, except at the solitary mid-rib.

The cambial strands of the glumes are the first to appear in the spikelet, and are present in very young rudiments, which have not yet half encircled the axis, that is, as soon as the spikelets are distinguishable from the ends of the branches of the panicle. No sections were cut at an earlier stage, but it is probable that the strands are present in the axis before the rudiments have appeared, as in the case of the mid-rib of the vegetative leaf and in the lemma. The upward extension of the strands keeps pace with the elongation of the glumes, and when the glumes are about half their mature length,

lignification begins at the base of the glumes. There is no trace of any lateral strands in the lower glume, or in the axis below it, but in the upper glume a lateral nerve appears on each side (Figs. 3, 4 and 5) very soon after the mid-rib and extends for a short distance into the glume base, but its growth is soon arrested. These minute strands become lignified shortly after the mid-rib but can hardly be of importance in translocating water to the mesophyll as they scarcely extend above the insertion of the glume.

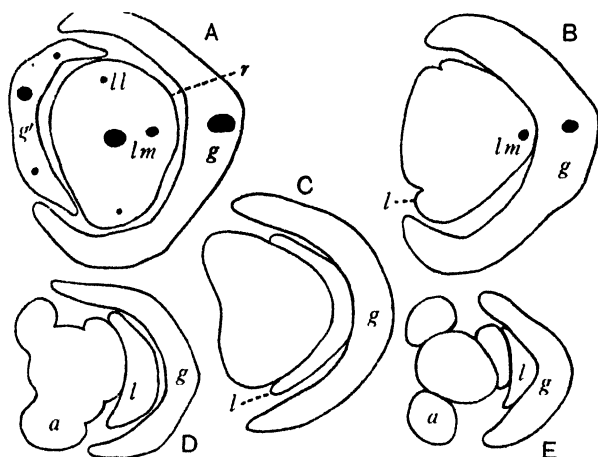


Fig. 3. Transverse sections from below upwards through a spikelet at the stage shown in Fig. 2 D a, anther rudiments, g, g', lower and upper glumes; l, lemma rudiment, lm, ll, mid-rib and lateral nerves of lemma; r, rachilla. All $\times 225$.

The lemma

The rudiment of the lemma encircles the rachilla more completely than those of the glumes, but like them its margins do not fuse. Growth in circumference takes place in the lamina as it increases in length so that the margins just overlap (Fig. 5). The lemma therefore completely wraps in the stamens until the time the flower opens. In the description of the external morphology of the young spikelets it was stated that the rudiment of the awn appeared on the back of the lemma near to the apex. It is possible to follow the initial development of the awn more closely in serial sections. When the apex of the lemma has just grown above the level of the top of the stamens the central cells of the top, which are still meristematic, develop a papilla upon the adaxial side. This papilla and the apex of the lemma develop independently at their apices in a parallel

direction (Fig. 5 G, H, I). The papilla gives rise to the awn and the lemma continues its growth, chiefly by divisions of its upper cells, as the insertion of the awn is only raised slightly higher above the insertion of the lemma (Fig. 2 E, F) before the cells become vacuolated, and the mature size is attained. Sections through younger

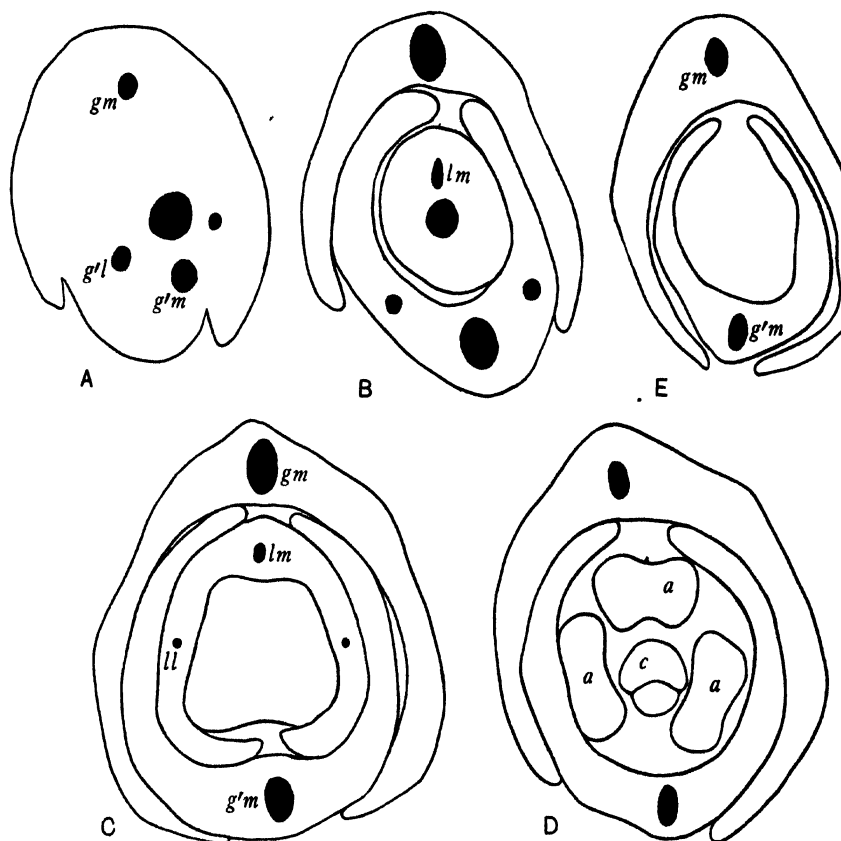


Fig. 4. Transverse sections from below upwards through a young spikelet just before the formation of the awn. *c*, carpel, *gm*, *g'm*, mid-ribs of lower and upper glumes; *g'l*, lateral nerve of upper glume; other lettering as in Fig. 3. All $\times 225$.

lemmas of *A. canina* show no trace whatever of the awn (Fig. 4) and are indistinguishable from lemmas of a similar age in *A. tenuis* and *A. stolonifera*. The awn rudiment does not at once develop rapidly in length, being for a time shorter than the lemma, but eventually its cells increase greatly in size and become highly vacuolated, and the awn quickly exceeds the glumes in length.

The mesophyll of the lemma is more extensive than that of the glumes, probably because of the greater number of nerves, and extends nearly from one margin to the other, almost to the apex. The epidermal cells on the back of the nerves, on the awn, and at the apex and the margins become toothed, and a close and uniform asperulence develops on the back.

The cambial strand of the mid-rib of the lemma is present in the axis before the rudiment is discernible. It is soon followed by a pair of lateral strands (Fig. 3), which also form in the axis, and then a marginal pair (Fig. 5). The lateral nerves appear before the marginal although they do not develop so high into the lemma and are weaker than the laterals. In *A. tenuis* the laterals are usually absent. The mid-rib develops upwards and becomes deflected into the awn. The laterals develop in a ridge of tissue in each side of the awn and the marginal strands in a thickening where the margins of the lemma are deflected sharply inwards. The marginal and often the lateral nerves are shortly excurrent as fibrous cells, but the lignified tissue is very scanty and confined to the basal parts of the strands.

The palea

The palea is very minute in the mature spikelet of *A. canina*, but appears as early in the young spikelets of this species as in *A. tenuis* and *A. stolonifera*, in which it attains half the length of the lemma or more. The base of the rudiment extends to only half the circumference of the rachilla in all the species, being stopped by the lodicules which arise at practically the same level (Fig. 5C). In *A. canina* the upper part does not increase in circumference as does the lemma, and only a slight increase takes place in the species with a larger palea. The palea occupies the space between the bases of the two anterior stamens and increases in length as the filaments appear. In its early stages it is similar to the palea of *A. stolonifera*, that is it does not lag behind in development, but its growth is soon arrested, so that it remains minute in the adult spikelet.

The palea is usually considered to be the first leaf of the floral axis, and as in the prophyll of the vegetative shoots it is two nerved (Fig. 5 B, C). The cambial strands appear about the same time as the marginal nerves of the lemma; they do not extend far into the lamina and no lignification was found in any of the spikelets.

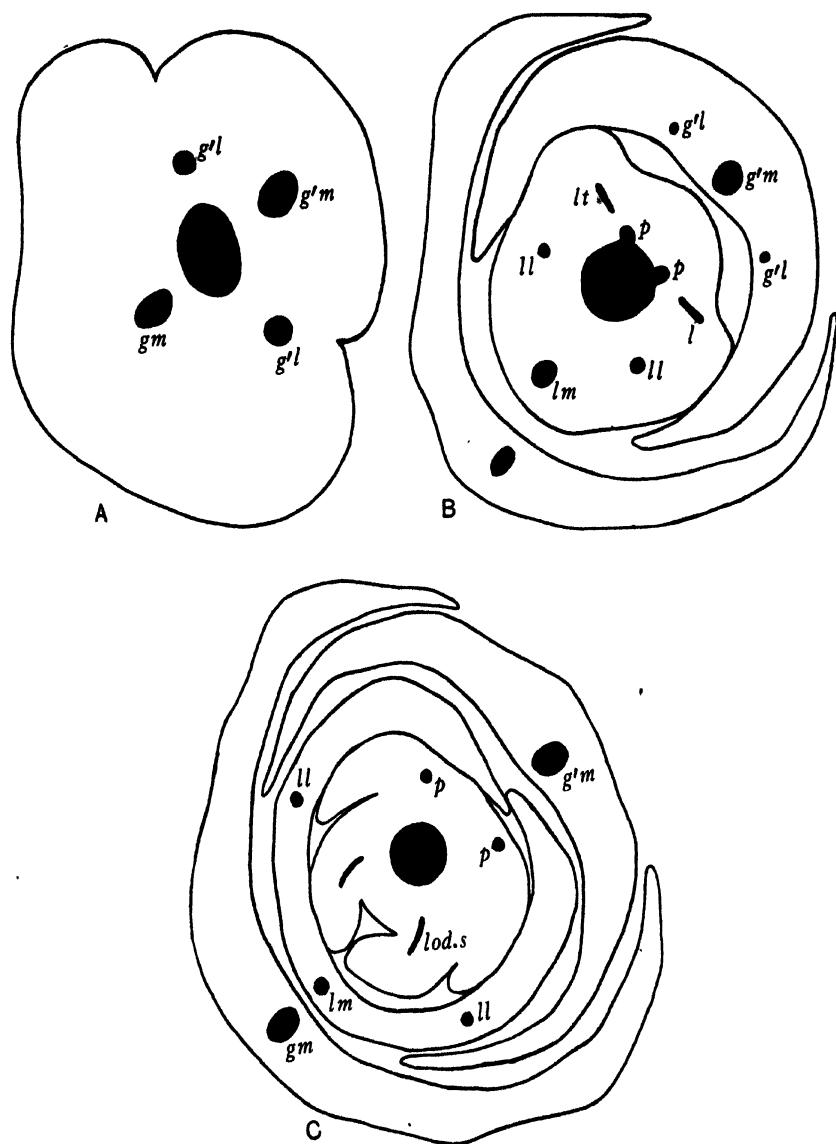


Fig. 5. Transverse sections from below upwards through a young spikelet just after the formation of the awn. *ll*, marginal nerve of the lemma; *p*, *p*, nerves of the palea; *lod*, lodicules; *lod.s*, lodicular strand; *d*, awn rudiment; other lettering as in Fig. 3. All $\times 225$.

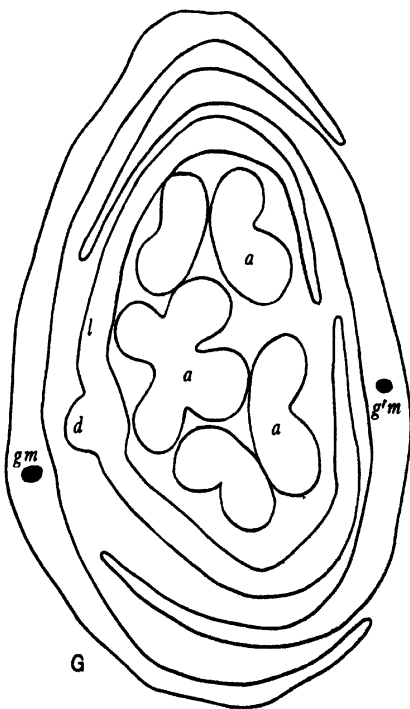
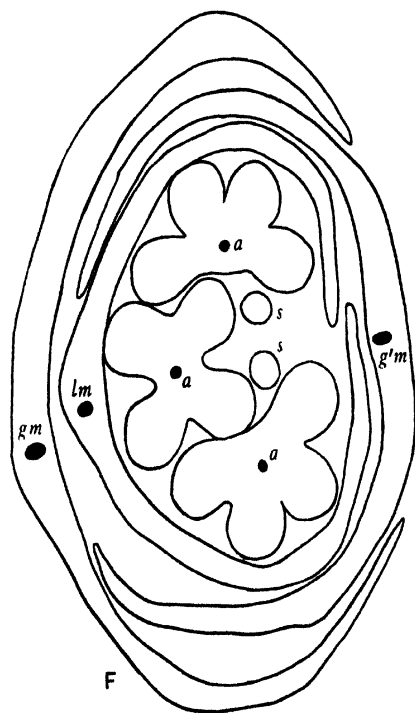
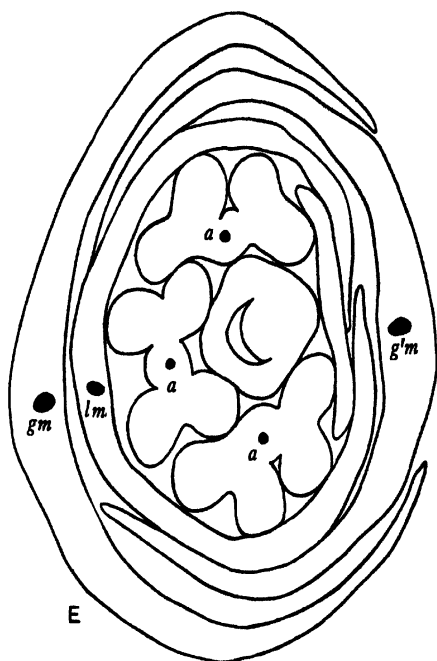
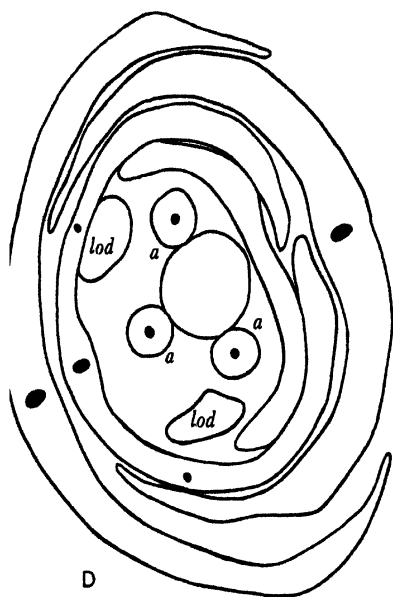


Fig. 5 (continued).

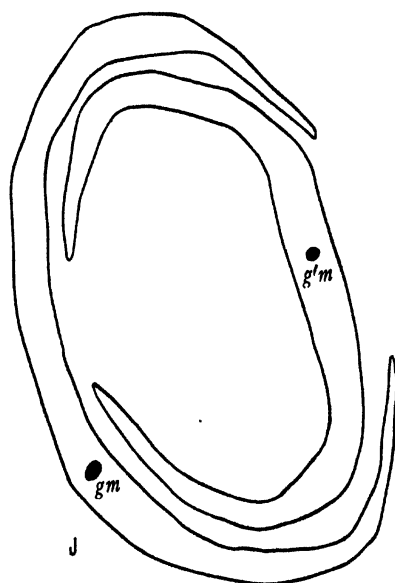
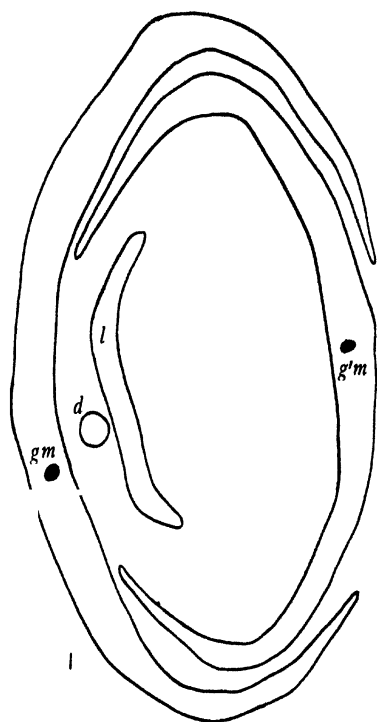
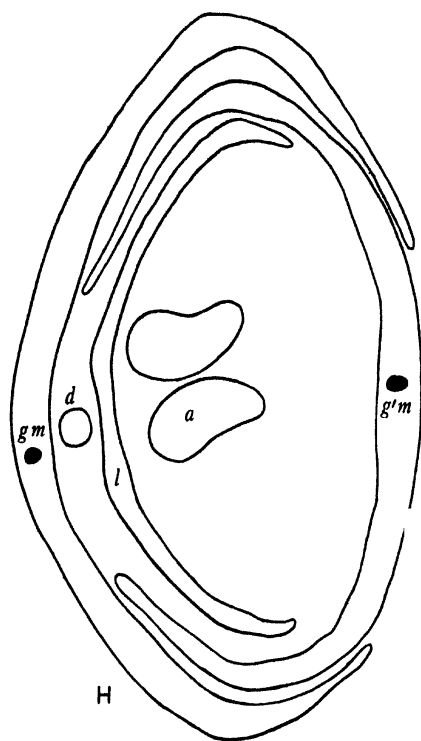


Fig. 5 (continued).

The lodicules

The rudiments of the two lodicules appear at the same level as the palea, and occupy the space between the posterior and the two anterior stamens (Fig. 5 C, D). Their bases become fused together so that the mature lodicules appear as a single bifid organ, and their margins become fused to those of the palea before its insertion, forming a very short cylindrical sheath around the floral axis. As the filaments of the stamens elongate the lodicules continue to grow, usually being about twice the length of the palea in the mature spikelet. Each is provided with a single cambial strand (Fig. 5 C) which becomes more strongly developed than those of the palea, although it appears slightly later.

The stamens

The three stamens form as spherical swellings symmetrically about the apex of the floral axis (Fig. 3 D). These soon become oblong and four longitudinal furrows appear. The epidermal layer is very distinct, and the four strands of sporogenous tissue stand out in the sections from the earliest stages, because of their deeply staining contents. The outer cells of the sporogenous tissue form a definite layer between the epidermis which divides twice to form four layers around each group of spore mother cells. These four layers are formed by the time the anthers are completely enclosed in the lemma and just before the filaments begin elongating.

A single cambial strand forms in the connective of each stamen (Fig. 5 D) immediately after the lodicular strands are laid down. These strands reach to the top of the anthers and become more strongly lignified than those of the palea and the lodicules.

The carpel

A fold of tissue grows up on the anterior side of the apex of the floral axis (Fig. 4 D) just after the stamens have become spherical. It continues to grow very slowly, curving over the apex of the axis and fusing with the adaxial side (for figures see Payer (1857), Pl. 148, figs. 24-8). The ovule develops from the enclosed apex. The carpel becomes closed at the stage when the four layers are distinct around the sporogenous tissue of the anthers. Two styles develop from the top of the fold (Fig. 5 F) and later become branched and develop a stigmatic surface.

The central strand of the floral axis enters the base of the ovule,

and is the only nerve of the carpel. Other grasses often have three nerves in the wall of the carpel, corresponding, according to Arber (1934), to the mid-ribs of the three components of the carpel, but these are quite absent in *A. canina* in which the vascular supply of all the parts of the spikelets is very meagre.

THE MORPHOLOGY OF THE DORSAL AWN

Several authors figure immature spikelets of species in which the lemma bears a terminal awn. As seems inevitable the awn develops from the first part of the lemma rudiment to appear, and usually elongates considerably before the lamina of the lemma is greatly developed. Duval-Jouve (1871) based his classical interpretation of the dorsal awn as equivalent to the petiole and blade of the vegetative leaf on the development of the awn in *Stipa*, in which the awn is not only terminal but three nerved, and undoubtedly equivalent to the apex at least, and perhaps all of the blade region, of the lemma. It is hardly valid to assume from the early appearance of the terminal awn, which cannot be disputed, that of the dorsal awn, so that without additional evidence the homology of the dorsal awn must be uncertain.

An account of the development of the lemma of *Avena fatua*, which bears a dorsal awn, was given by Cannon (1900). The main part of his paper deals with the development of the stamens and the carpel, but he also described the origin of the bracts. In the summary he states that "the awn of the lower glume [lemma] appears before its lamina, the latter being an outgrowth from it. Thus the origin of the parts of the lower glume resembles that of the blade and the ligule of the vegetative leaf". He gives a figure of the lemma (Pl. XLIX, 2) at a stage when the lamina is a small ventral outgrowth of the awn.

The development of the lemma in *Agrostis canina* as described in the present paper does not agree with Cannon's account for *Avena*. The lamina forms just as it does in the awnless species of *Agrostis*, and the awn appears at a late stage when all the five cambial strands have been laid down. The awn forms by a division tangential to the floral axis of the apex of the lemma, the two apices so formed continuing to develop on parallel courses, one as the awn, the other as the lamina. The ligule of the vegetative leaves does not develop in this way but as an outgrowth at the base of the young leaf-blade.

The transitional structures between the lemma and the leaf found

Development of the Spikelet in Agrostis canina L. 435

in proliferating spikelets of *Deschampsia caespitosa* (Philipson, 1934) supports the view that the dorsal awn is not equivalent to the whole of the blade, but only to part of it. In *Agrostis stolonifera* (Philipson, 1935) the whole of the apex of the lemma with its five nerves is included in the blade of the leaves developed in proliferating spikelets. The apex of the lemma in *A. canina* and *A. stolonifera*, whether in their awned or unawned forms, must be of the same morphological nature, that is it represents the blade region of the leaf.

Since Cannon was not primarily concerned with the development of the bracts of the spikelet but with the floral organs, and since his figure of the young lemma is rather unconvincing, I do not think it can be certain that the development of the lemma in *Avena* is materially different from that in the closely related *Agrostis*. The development of the awn in *A. canina* supports the interpretation already put forward (Philipson, 1934) that the dorsal awn represents part of the blade which has become separated as a dorsal outgrowth, the main part of the blade remaining as the upper part of the lamina. A terminal awn, which is actually the attenuated apex of the blade, or even the whole blade region, would naturally appear first in the rudiment of the leaf.

SUMMARY

1. A brief account is given of the formation of the branches of the panicle.
2. The development of the young spikelets is described as seen as solid objects under the binocular microscope.
3. The initiation and development of the bracts and floral organs with their vascular supplies, as they can be made out from microtome sections, are described.
4. The morphology of the awn is discussed, and it is maintained that the awn represents a part of the blade which has become separated as a dorsal outgrowth.

My thanks are due to the Department of Scientific and Industrial Research for a grant to enable me to carry out work on the British forms of *Agrostis*, and to the Director of the Royal Botanic Gardens, Kew, for permitting me to work in the Jodrell Laboratory.

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NOTE ON EXUDATION AND EXUDATION PRESSURES IN BIRCH

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(With 2 figures in the text)

THE recent work of James and Baker (1933) has again brought the question of "bleeding" and exudation pressures into prominence by suggesting that the sap exuding from a cut branch or root is transmitted through the living cells, probably the sieve tubes of the phloem, and not through the xylem vessels as has generally been supposed. The point of view of these workers can best be given by quoting from their paper. They state: "It is thus clear that, although a possible exception may have to be made for vine branches, there is no sufficient evidence to justify the assumption that exudations usually well out of the vessels." And again: "In view of these facts it seems preferable to suppose that root and stem exudation pressures are transmitted, not through the vessels as usually assumed, but through the living symplast, and that any movements of liquid due to them take place principally through the sieve tubes." James and Baker base their conclusions on experiments carried out with sycamore.

In connection with this problem some observations made on the "bleeding" of birch trees may be of interest. Amongst British plants *Betula alba* appears to exhibit the phenomenon of "bleeding" to a greater extent than in any other tree. It must be emphasised that in the intact plant little or no movement of sap occurs as the result of the exudation pressure developed and that it is only when a branch is cut that free flow can occur in the conducting system. During the period when active exudation may be observed, which lasts usually from early March until the bursting of buds in April, sap drips steadily from cut shoots and stems. Early in the bleeding season sap flows only from cuts made in the roots or in the lower branches of the tree and at this stage the rate of flow from comparable branches decreases with height above the ground. Later, however, a cut in any part of the tree, provided the wood is injured, leads to "bleeding". Even at the top of a tree 11 metres high active exudation was observed.

"Bleeding" is so vigorous during the period of maximum activity, towards the end of March, that sap can readily be collected by the litre. Two cases may be quoted to illustrate the rapidity of exudation. A cut branch 1.5 cm. in diameter exuded sap at the rate of 25 c.c. per hour maintaining this rate for 4 hours. This represents quite a slow flow for birch. The most rapid exudation observed was from the apex of a young tree 2.5 m. in height. When the tip was cut off 8 c.c. exuded in 25 min. although the diameter of the cut stem was less than 2 mm. From a cut branch "bleeding" may continue for weeks, although the actual rate of exudation at any time depends on meteorological conditions. There is a tendency to a diurnal periodicity, exudation falling to a minimum or stopping altogether in the late evening.

When a thick lateral root is severed exudation usually occurs from both the cut surfaces, although the rate of "bleeding" in a downward direction is almost always greater than in an upward direction.

Shortly after the bursting of the buds exudation of sap can no longer be detected.

Observations indicate that "bleeding" only occurs if the wood is damaged. This can most readily be shown by carefully cutting into the trunk of a large birch tree. Immediately the wood is cut the sap flow starts. Bleeding from the wood was demonstrated conclusively by the arrangement shown in Fig. 1. A branch near the ground level was cut off and the end of the stump cut into the form of a tapering spike so that no tissue external or internal to the wood contributed to it. To the end of this spike a rubber tube was fixed and to this a graduated glass tube could be attached when an observation of the rate of "bleeding" was desired. It is worth noting that no wood younger than 2 years formed part of the end of the spike

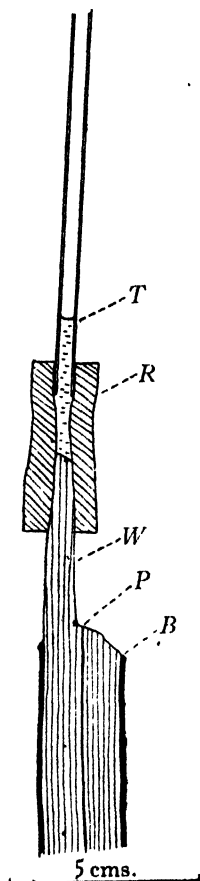


Fig. 1. Stump of a side branch of birch (see text) shown in L.S. T, graduated tube; R, pressure tubing; B, bark; P, pith; W, wood. Annual rings of autumn wood shown by thin black lines.

to which the tube was attached. Table I gives some records of the rate of "bleeding" from this spike.

TABLE I

Date	Time of observation	Rate of "bleeding" from spike (Fig. 1) in c.c. per hour
March 29th	12.52 p.m.	0.76
" 29th	3.50 "	0.81
" 29th	8.30 "	0.33
" 30th	10.2 a.m.	0.85
" 30th	2.52 p.m.	0.46
" 31st	9.15 a.m.	0.75

Similar results were obtained using both lateral branches and roots.

Some observations along similar lines were also made using stumps of recently felled sycamore trees. In these "bleeding" from the wood was clearly shown in early April. Fig. 2 is an accurate longitudinal section of a stump on which a small projecting spike of oldish wood was isolated. A vertical capillary tube (1.0 mm. bore) 88 cm. in length was attached to this with a rubber connection. After several days the sap had risen to the top of the tube and had overflowed.

If the sap moves in the xylem it may either flow through the dead or the living part. No direct evidence is available on this point at the moment. In the case of birch, however, it is difficult to believe that such a rapid movement of sap as that which occurs when a branch is cut could take place except through the dead, water conducting elements of the xylem, since the resistance to flow in the living cells is probably very considerable and the exudation pressure is rarely much more than 1 atmosphere.

In the introduction to their paper James and Baker state: "All the more important attempts to elucidate these pressures have centred upon the problem of explaining the passage of water from the living parenchyma into the vessel cavities, and a proof that no such passage need occur would necessarily be of great importance to the theory of the subject." However, in the simple theory of root pressure put forward by Atkins (1916) the passage of water from the living parenchyma into the xylem vessels presents no difficulties. According to Atkins: "The solution in the tracheae acting osmotically through the semi-permeable membrane formed by the outer tissues of the root determines a flow of water from the soil to

the tracheae, and the resulting hydrostatic pressure is responsible for the exhibition of bleeding and root pressure characteristic of spring." On the theory of James and Baker the osmotic pressure of the exuding sap would bear no relationship to the exudation

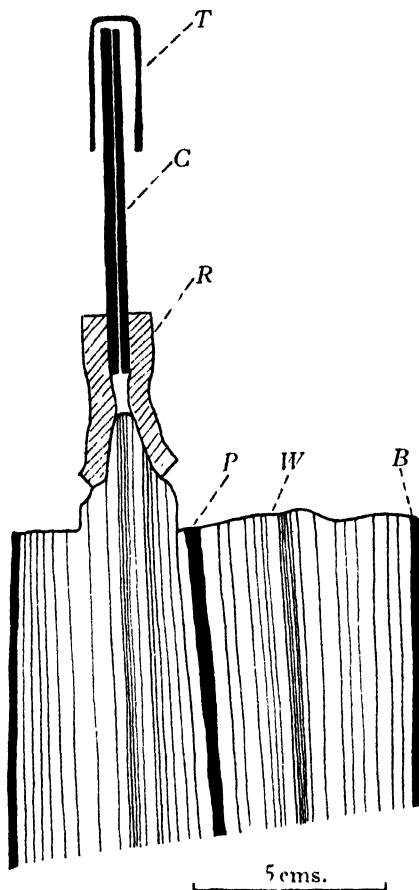


Fig. 2. L. S. of sycamore stump referred to in text. T, inverted specimen tube; C, capillary tube (this was ten times as long in the actual experiment); R, pressure tubing; B, bark; P, pith; W, wood. Annual rings of autumn wood shown by thin black lines.

pressures developed. On Atkins' theory, however, the osmotic pressure of the exuding sap, or at any rate the sap pouring from a bleeding root, might be expected to be equal to or greater than the exudation pressure developed. Simultaneous measurements of the exudation pressure and the osmotic pressure of the exuding sap do

not seem to have been made, so some observations on this point were collected using birch trees growing on a heath near Reading. In each tree a branch about 30 cm. above the ground level was cut off, a mercury manometer attached to the stump and the exudation pressure measured. The pressure gauge was then removed and a sample (usually about 20 c.c.) of the exuding sap was collected. In some cases a root of the same tree was also cut and sap collected from the exposed surface. The osmotic pressure of the sap was determined cryoscopically. Although both the exudation pressure and the osmotic pressure of the sap varied considerably from tree to tree, in no case (Table II) did the exudation pressure exceed the osmotic pressure.

TABLE II

No. of tree	Osmotic pressure of exuding stem sap in atmospheres	Osmotic pressure of exuding root sap in atmospheres	Exudation pressure in atmospheres
1	1.44	—	0.47
2	2.40	2.64	1.64
3	1.68	—	1.44
4	1.92	—	0.79
5	2.16	1.68	0.53
6	1.44	1.20	0.49
7	1.68	1.68	0.55

The evidence for birch, in so far as it exists at the moment, appears to support Atkins' view of exudation pressures rather than the more recent theory of James and Baker. It must be remembered, however, that the mechanism of exudation may not be the same in all plants.

It is hoped during the next few years to make a detailed analysis of "bleeding" in birch with a view to elucidating the mechanism involved.

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KRAKATAU AND ITS PROBLEMS

BY W. B. TURRILL

THE flora and vegetation of islands must always be of particular interest to phytogeographers and ecologists, since problems of origin and development are in some respects simpler and in others more difficult than for land-locked areas. The history of Krakatau since the great eruption in 1883 is familiar to English students largely because of Prof. Seward's translation (Cambridge University Press in 1908) of Prof. A. Ernst's account published in Zürich the previous year. A more recent account by C. A. Backer (published by the author, 1930) was also written in English. This latter is a book of three hundred pages under the title *The Problem of Krakatao as seen by a Botanist*. Backer, in opposition to Treub, Ernst, and most other scientists who have visited the main island since the catastrophe, concluded that it has not been proved, and that it is even improbable that the old vegetation was totally destroyed by the eruption of 1883. Backer's arguments are weakened by a dogmatic and unnecessarily polemical style, which, rightly or wrongly, leads the reader to suspect a personal bias of the writer against one or more of the previous investigators. There remains, however, a considerable mass of facts and statements which have to be considered by those who believe that a large and unique example of primary succession can be traced in the new vegetation of Krakatau.

Prof. Ernst has now published (*Vjschr. naturf. Ges. Zürich*, **79**, 1934) a paper of 187 pages on "Das biologische Krakatauprobem", in which the whole problem is carefully reviewed, data from still more recent investigations are added, and Backer's arguments at least partly met. The paper is illustrated by seven plates and five text-figures and has five and a half pages of references.

There can be no doubt that the existing growing vegetation of what is now Krakatau and the associated smaller islands of Verlaten Eiland and Lang Eiland was mainly if not entirely destroyed in 1883. Backer, himself, thinks it most probable that the two smaller islands were completely denuded of living organisms. The crucial matters are whether or not some vegetation survived in ravines on the higher parts of Krakatau, especially on those sides not visited by Treub in 1886, and whether on the higher parts of the island the ash covering

was sufficiently hot and remained everywhere long enough to kill all seeds and underground plant parts such as deeply seated rhizomes. It is obvious that, half a century after the event, only the statements of earlier investigators and indirect circumstantial evidence are available. The earlier biological investigators were unanimous in concluding that all living organisms were destroyed and that the new flora and fauna immigrated from surrounding land. Their judgment was based on close investigation of sample areas, which though not so numerous or so extensive as is now known to have been desirable, were, from the standpoint of the main problem, chosen at random. Considerable weight must be given to the published accounts of Treub, Verbeek, and Penzig. The possible survival of seeds or other disseminules under a temporary ash covering on the higher slopes can only be considered by most indirect evidence. Ernst notes that plants with deeply seated rhizomes (Zingiberaceae, Musaceae, Araceae) are even now represented by relatively few species.

It is impossible in a short review to do justice to the evidence, so much of it being matters of detail. While it cannot be proved in any absolutely certain manner that every trace of living organisms was destroyed there can be no doubt that the three existing islands of the group have become clothed with an essentially new vegetation. The greater part of Krakatau itself must now have a vegetation which represents one or other stages of a primary succession. The investigations summarised by Ernst have therefore a peculiar value to ecologists. On the other hand, the problem of complete or incomplete destruction of the former vegetation is of less importance to students of plant dispersal than it would have been had Krakatau been much farther from surrounding land masses. Sebesi Eiland is only 19 km. from Krakatau (and less from Lang Eiland and Verlaten Eiland) and the vegetation on Sebesi Eiland was only partially destroyed. Parts of Java and Sumatra are only 40 km. distant. Transport of disseminules over much greater distances by the common agents of dispersal (birds, wind, water) has been proved by many independent observations in widely different parts of the world. Phytogeographically Krakatau is of less interest than it is ecologically.

Ernst summarises the existing plant formations, largely on the work of W. M. Docters van Leeuwen, as follows:

- (1) The Strand-Zone with the *Ipomaea pes-caprae* formation, and
- (2) The *Barringtonia* wood, both of unequal width and still (up to 1919) discontinuous.

- (3) The *Casuarina* wood here and there in extensive groups.
- (4) Mixed wood and *Macaranga* formation, over considerable areas with a strong development of ferns as undergrowth.
- (5) The Grass steppe, which in 1906 occupied the largest part of the island, still covers considerable areas.
- (6) The *Cyrtandra* formation on the higher slopes, with considerable richness in epiphytes.

In addition to coastal changes due to the removal and redeposition of the mobile strand terrain, with destruction of some of the pioneer vegetation, successional changes are summarised by Ernst. The *Casuarina* wood is the first woody vegetation to develop on the Krakatau islands, but is the one with the shortest existence, since once it has established itself it is invaded by and soon destroyed by elements of the *Barringtonia* community which over-shade the light crowns of *Casuarina*. Another striking successional change is the reduction of the grass steppe area. This formerly dominated in the inner coastal stretches and on the lower slopes of the peak but is being rapidly invaded by forest both from below and from the ravines of the mountain.

Unfortunately early investigators after 1883 did not reach the peak and changes in the *Cyrtandra* forest can only be recorded from 1908. It would appear that *Cyrtandra sulcata* has grown and multiplied rapidly to produce a rather low but thick wood, which, in habit but not in composition, is now similar to the forests on volcanic peaks in Java and Sumatra. It is probable that the *Cyrtandra* wood is not a climax at the relatively low altitude of the Krakatau peak and it may be expected that in the not far distant future a forest covering will be developed of the same luxuriance and composition as that destroyed in 1883.

REVIEWS

The Diseases and Curing of Cacao. By H. R. BRITON-JONES. Pp. vi+161. 37 figs. Macmillan and Co., London. 1934. Price 10s.

The present volume adds one more to the list of useful hand-books and manuals dealing with the diseases of tropical crops which have appeared from time to time during the last 15 years. The first was on Rubber by Petch and appeared in 1921. This was followed in 1923 by a volume on Tea also by Petch and then close together the present work and a fourth on the Banana by Wardlaw. All are published by the Macmillan Co. and are in the same style.

Prof. Briton-Jones' work on cacao is the outcome of a discussion on the further need for such handbooks which took place at the Imperial Mycological Conference held in London in 1929. In his preface the author states that he has written his book chiefly for Agricultural Officers and planters, and anyone who has experienced the difficulties and lack of information existing in the tropics where crops are grown on a commercial scale will at once commend such treatment. The great desire of either a planter or an Agricultural Officer is to be in a position to find out what is wrong with his crops, and having done that either to be able to cure the trouble or prevent it occurring in the future. The scientific names of particular parasites and details of their life histories he has no use for, and probably would not understand. His ever recurring enquiry is, "how can I get rid of this disease?" With this laudable aim in view Prof. Briton-Jones has given an account of all recorded diseases of cacao from the field point of view, stressing the naked eye characters by which they can be recognised, and outlining the control measures where known or suggested.

The book is necessarily a compilation of the author's own observations and investigations in Trinidad together with those undertaken in other countries, for unlike tea or rubber, cacao is grown in many parts of the tropics where neither the conditions nor the ways of handling the crop are the same. The end-product, the cacao of commerce, which is exchanged for what the planter really desires, namely money, is similar wherever it is produced. The manner of its production is not the same, and it cannot but add to the interest of planters and Agricultural Officers as well, to learn in what respects their methods differ from those in other countries. But here arises a dilemma. Should a book written primarily for planters and Agricultural Officers be arranged under countries and every disease known in that country dealt with in that section: or should the diseases of the various parts of the plant form the chief divisions with adequate references to the countries in which they are found? Either treatment entails repetition, the former requiring more than the latter, but this could be reduced to a minimum by cross-references. Prof. Briton-Jones has chosen the latter method thereby diminishing the usefulness of the book, for anyone, say, on the Gold Coast, has to look up at least 14 different pages for the various diseases in that territory.

The book consists of the following chapters: Root diseases, Stem diseases, in which are included those of the foliage, Pod diseases, Witches' Broom disease and a final chapter on the preparation or curing of cacao. There are two bibliographies, the first citing 40 works dealing with cacao fermentation, the second of 192 titles in connection with the chapters on disease which are also illustrated with 36 good reproductions of original photographs. Some of these, however, bear no indication of the scale used and while not essential for such objects as pods or leaves, the actual or average size of the fructifications

of *Ustulina zonata* and *Armillaria mellea*, so important for diagnosis are left to the imagination.

It was a happy thought to include a chapter on fermentation or curing, which is both interesting and stimulating; the author rightly points out the need for further biochemical research in this all-important subject. The excellent sentiments expressed in the preface are not always evident in the text and the very first chapter dealing with a root disease in West Africa contains not only a detailed account of the changes of the name of the fungus concerned, but also describes the disease as it occurs on another host, namely rubber, on which alone it is economically important in the East. The reason given is that "there is no full description of the disease on cacao available", which one would have thought to be an excellent reason for omitting it altogether. The author continues "it is applicable to cacao, since it is on similar descriptions that the disease has previously been identified on several host plants, including cacao". This is neither logical nor mycological, but the account will prove of interest to those concerned with rubber. Again in the same chapter the author has sadly fallen from the high standard set out in the preface of avoiding technical and detailed descriptions wherever possible "as of little or no use to either class of Agriculturist" by quoting in full Corner's botanical description of the fructifications of *Ganoderma pseudo-ferreum*.

Apart from his account of all the diseases recorded on cacao the author is perhaps most interesting in his discussion on the control of root diseases. It is only natural that he should dilate on the subject of his own investigations but the citations from his reports on Witches' Brooms might have been curtailed. The general impression one gets from this handbook is that there is a good deal more to be discovered about the diseases of cacao and how they may be controlled and this book should stimulate further research into these matters. It will certainly be welcomed by the harassed Agricultural Officer and planter and its price of 10s. is not excessive.

One would have liked to see more stress laid on the relative importance of the various diseases treated, as also of the comparative losses, entailed, and a table, or list, showing at a glance the most serious disease of any one country, would have been useful, particularly to those for whom the book has been specially written.

W. J. DOWSON.

A Flower Book for the Pocket. By MACGREGOR SKENE. $5\frac{3}{4} \times 4\frac{1}{4}$ in.

Illustrations by CHARLOTTE GEORGIANA TROWER and RUTH WESTON. Pp. 380, illus. 529. Oxford University Press, 1935. Price 7s. 6d. net.

This little volume will undoubtedly fill a gap in the list of works on the British flora, and should appeal to a wide public of amateur flower lovers. It is botanically more advanced than the majority of popular floras, yet it is considerably easier for the unskilled to use than, say, Hayward's *Botanist's Pocket Book*. This intermediate standard, together with its pocket size and profuse illustrations, are the features which constitute its distinctive appeal.

The opening chapter, entitled "How to use this Book", includes a full explanation of the various botanical terms used, and contains enough information to enable a complete beginner to find his way through the pages that follow. The next two chapters are devoted to keys to families and genera. These are excellent. Tests with half a dozen different species proved entirely successful. The author has wisely used simple and easily seen characters wherever possible, thus avoiding the somewhat terrifying effect of the highly technical keys that confront the beginner in works of a more advanced character.

The main part of the volume contains descriptions of 529 of the commoner or more striking species in the British flora, each illustrated in colour, together with short descriptive notes on 315 of the rarer or more critical species, making the total number of species described 844. On the whole the descriptions are accurate and quite adequate for identification. The determination to have every plate opposite the description of the species, which is in many ways admirable, has made the volume much bulkier than was really necessary with the present number of species, as on many of the pages there are blank spaces. If another edition is called for, it would be wiser either to increase the number of species mentioned, or to print the plates apart from the descriptions and thus save space. The former course would probably be the more acceptable to many readers, as several genera could with advantage be more fully represented. Thus only eight *Carices* are mentioned, though there is ample space for as many again; a place might well be found for *C. riparia*, *C. praecox*, *C. hirta*, *C. paniculata* and *C. acutiformis*.

The selection of species for a work of this type is to some extent subjective, and other botanists are certain to find omissions or inclusions which are not to their liking. For example, no mention is made of the exceedingly common *Rumex nemorosa* (*R. condylodes*), though the much rarer *R. sanguineus* is included, and surely *Poa trivialis* might have been vouchsafed a line along with *P. pratensis*?

The position of the accent is shown in the Latin names, a feature that should help to raise the very low standard of British botanical pronunciation. The stress in *Anemone*, however, is shown on the second instead of on the penultimate syllable.

The great majority of the coloured plates are by the late Miss C. G. Trower, and have been placed at the disposal of the publishers by the executors of the late Dr G. C. Druce, to whom they were presented by the artist.

The success of the process of reproduction used, by which the plates are printed direct on to non-glossy paper, is very uneven. On the whole, the soft tones and rather blurred outline produced are pleasing and give a fair representation of the species—especially in purple and red flowers. Yellow and blue flowers are less good, while some of the white species are completely unrecognisable— notably *Sagina nodosa* (No. 80), which, owing to there being no indication of size, looks like a large blue *Linum*, *Stellaria uliginosa* (No. 76), and *Arabis hirsuta* (No. 30). In a second edition, the opportunity might be taken to improve the reproduction of the plates, the originals of which must be a very delightful set of water-colours.

The format and price are the same as those of the earlier *Bird Book for the Pocket*, and the present work should certainly be as popular with amateur botanists as was the previous volume with bird lovers.

J. S. L. GILMOUR.

The Structure and Reproduction of the Algae. Vol. I. By F. E. FRITSCH, D.Sc., F.R.S. $8\frac{1}{2} \times 5\frac{1}{2}$ in. Pp. xvii + 791, with frontispiece and 245 text-figs. Cambridge University Press, 1935. Price 30s. net.

This is vol. I of Prof. Fritsch's book on the morphology of algae, the first of its kind ever to be published in English. It is the more welcome because the vast body of new facts and new ideas which has emerged since the publication of the second edition of Oltmanns' great work has received until now no adequate summary and documentation. Prof. Fritsch has made splendid use of his opportunity and will relieve teacher and pupil alike of much hard reading in foreign books and periodicals, while giving them in addition all the benefit of his experience and judgment.

The volume opens with a valuable discussion of the relation between Flagellata and Algae. It is concluded that the distinction cannot be maintained and that the former term should therefore be dropped, the term "alga" being used to "include all holophytic organisms (as well as their colourless derivatives) that fail to reach the higher level of differentiation characteristic of the archegoniate plants". The flagellate theory is then stated and the now familiar idea expounded of several more or less parallel series of algae, differing chiefly in physiological characters. The chief characteristics of the eleven main series are listed, and the remainder of the introductory chapter deals in a general way with the structure, reproduction and life history of algae. There follow chapters dealing in detail with eight of the main algal series, and an appendix on the "residual colourless flagellata". Each has a full bibliography, and there are 245 pages of figures taken, for the most part, from previous publications. Detailed accounts of the Myxophyceae, Phaeophyceae and Rhodophyceae will occupy the second volume.

Of considerable interest are the accounts in the introductory chapter of recent work on the cytology of algae, on their centrosomes and flagellar apparatus, their eyespots and vacuoles. The problem of sexual secretions has also become interesting but evidently is still in a very confused state. A brief summary of our knowledge of life cycles amongst algae emphasises the great diversity in this respect even amongst the morphologically less elaborate types. One becomes impatient to discover from the second volume why Prof. Fritsch regards the Fucal life history as a reduction product from the Laminarian.

It would be good to know more about the external covering of algae. Prof. Fritsch distinguishes a periplast from a wall by the fact that only the former normally divides with the protoplast. In dealing with motile unicellular Dinophyceae he talks of an "envelope", and his difficulty is obvious, since in some species of *Peridinium* this envelope does not rupture at cell division, but grows "over the surface of the dividing protoplast *pari passu* with its fission", while in other species it is ruptured and discarded, the daughter cells acquiring complete new envelopes of their own. *Ceratium* shows an intermediate condition, in that the two daughter individuals have each a half of the parental envelope and grow other new halves after separation. Placoderm Desmids—and Diatoms—behave rather like *Ceratium*, but *Chlamydomonas* like most species of *Peridinium*. Clearly periplast and wall are not sharply distinguishable, at any rate in their behaviour during division. Perhaps this is determined primarily by the rigidity and thickness of the envelope and is of no fundamental significance. The observation of Pascher, quoted by Prof. Fritsch, that even in filamentous types which he has studied there is a complete new wall round each daughter protoplast, is a further complication. It is by no means clear, however, that this is true of all filaments, and may be exceptional. Careful observations of cell division in a wide range of types are badly needed and might give much valuable information as to the probable mode of evolution of the more elaborate types of thallus. It might be shown, too, that the mode of cell division in some series is directly related to the fine structure of the walls and this to their biochemical composition.

Other sections of the introductory chapter which makes one want to know much more are those dealing with more advanced thallus types. How, exactly, are the thalli of *Coleochaete scutata* and the *Culleriales* formed by "congenital fusion" of filaments? Does the term mean the same in both cases? A Baumechanik of higher algae, founded on close developmental studies, would be an interesting and instructive subject.

There is a wealth of information in the nine chapters of detailed description which summarise existing knowledge of the lower algae, and there are exhaustive bibliographies for the more serious student. Prof. Fritsch had already in earlier publications aroused our interest in series less familiar than the Chlorophyceae, and here at last it is possible to read in English an account of the whole fascinating diversity of form and behaviour in the Chrysophyceae, Dinophyceae,

Cryptophyceae and the rest. It does not matter that zoologists may reasonably claim many of these organisms as animals, for their relation to pigmented types is made manifest; and it is good that the botanist should be able to go at least a short distance in both directions from No-man's-land.

The adoption of Allorge's Xanthophyceae for Heterokontae and of Bacillariophyceae for the diatoms gives a pleasing approach to uniformity in the names of the series. But it seems odd that Prof. Fritsch should argue so convincingly that no sharp line can be drawn between flagellates and algae, and yet should deny the ending -phyceae to Euglenineae and Chloromonadineae merely because no algae representatives have yet been found.

The merging of Siphonocladiales with Siphonales and the erection of a separate order for Cladophora and its allies are useful features of the classification adopted. The inclusion of Charales in the Chlorophyceae is perhaps a logical outcome of the biochemical classification of algae, but it means that the various orders of Chlorophyceae are of very different status when Volvocales, Chlorococcales, Ulotrichales, Cladophorales, Chaetophorales, Oedogoniales, are set alongside Siphonales, Conjugales, Charales.

The frontispiece is of *Draparnaldiopsis indica*, one of the species of a recently discovered genus of Chaetophorales of very elaborate thallus construction. Its choice is significant, for Prof. Fritsch has frequently stated his faith that amongst this group of algae the beginnings of the land flora must be sought.

A. R. CLAPHAM.

International Rules of Botanical Nomenclature. 3rd ed. Pp. xi+152.
Jena, Gustav Fischer, 1935. 7 Rmk.

This third edition of the *International Rules of Botanical Nomenclature* was compiled by an Editorial Committee from the Report, by the late Dr J. Briquet, of the Subsection of Nomenclature of the Cambridge Congress, 1930. The original English text was drawn up by Dr Rendle, and German and French translations by Dr H. Harms and B. P. G. Hochreutner respectively. As compared with the second edition the number of principles and rules has been increased from 58 to 74, and of recommendations from 38 to 50. There are also important changes in the arrangement and wording of items.

There are four chapters dealing with General Considerations and Guiding Principles, Taxonomic Categories, Names of Taxonomic Groups, and Interpretation and Modification of the Rules. The third chapter is by far the longest and most important. It opens with a section stating such general principles as that of priority, and then follows a new section dealing with the "type-method" of nomenclature, whereby the name of a taxonomic group has a nomenclatural type permanently attached to it, thus determining its application in the event of the group being subsequently divided. It is recommended that this method be adopted in all future publications of names, while an appendix of proposed type-species for Linnaean generic names and for *nomina generica conservanda* takes an important step towards making the method applicable also to names already published. The remaining sections of this chapter contain the rules of limitation of priority, of nomenclature of the various taxonomic groups, of conditions of effective and valid publication of names, of citation of authors of names, of choice, rejection, orthography and gender of names, etc. There are two valuable appendices, one a list of *nomina generica conservanda*, the other a set of "rules" for the nomenclature of garden plants.

One cannot fail to be impressed by the persuasive reasonableness of most of the principles and recommendations. Those dealing with the formation of new names are specially noteworthy. It is to be hoped that a better standard of nomenclature and a closer approach to the high ideal of absolute uniformity will result from their perusal. Articles 36-45, dealing with the conditions of effective

and valid publication of new names, should be read carefully by the editors of journals publishing taxonomic papers as well as by all taxonomists.

It is interesting that certain family names, such as Gramineae, Labiatae, "sanctioned by long usage", are admitted although they do not end in -aceae, but that botanists are authorised to use as alternatives the appropriate names ending in -aceae. This seems an unnecessary exception to the rule that each group can bear only one valid name. And it seems unfortunate that suborders and subtribes should continue to have the almost identical endings -ineae and -inae respectively.

Many curious problems confront the student of nomenclature, and this volume gains in general interest by giving actual examples of the application of the principles and rules. The name *Actinotinus* must be rejected because it was based on a specimen of an *Aesculus* whose terminal bud a native Chinese collector had replaced by an inflorescence of *Viburnum*. *Ifloga* is a deliberate anagram of *Filago*, and *Thuspeinanta* of *Tapernanthus*. Errors in the gender of Greek derivatives must be corrected if perpetrated only by Linnaeus, Robert Brown, or Bentham, but if, like masculine *anthos* and *gaster*, they are more widespread, they are to be admitted. *Taonabo* is feminine because its author said so, and *Manthot* because Crantz decided to make it so, in the absence of guidance from its author.

The British ecologist will note that *Fagus sylvatica* must retain the spelling given by Linnaeus, but that in new names the adjective must have classical *i* for medieval *y*. He will note also that *Pteridium aquilinum* occurs in a supplement as a proposed *nomen conservandum*.

A. R. CLAPHAM.

Chronica Botanica. Vol. I. Ed. by F. VERDOORN. $9\frac{1}{2} \times 6\frac{1}{4}$ in. Pp. 447, with numerous illustrations. Leiden, 1935. 15 guilders.

There are, we are told, 1000 periodicals concerned with pure and applied botany, about 4000 institutions and nearly 70,000 botanists. It is the business of this thousand and first periodical to help co-ordination and to "keep you in touch". Its method is not that of the abstracting journal. Turning its pages one might feel that a more descriptive title would have been *Chronica Botanicorum*, subtitled by "the lives of the botanists". The personal touch is the thing.

The work is divided into three major sections. The first deals with Conferences, including a history of the International Botanical Congresses, and all botanical and allied meetings to be held during the current year. The second section gives "all the scientific and personal news", and includes an exhaustive list of addresses of botanical institutions, and photographs of members who died last year. Since the items were contributed voluntarily by the heads of institutions the entries naturally vary greatly in scope and degree of detail. Finally, there is a mixed section of correspondence, new periodicals and emendations to the International Address Book.

It would not be easy to prophesy the future of this well-advertised, well-executed and lavishly produced journal. It occupies a field hitherto vacant and of admitted importance. If eventually botanists learn to turn to its pages to find who shares their technical interests and where, a considerable service will have been rendered to botany.

W. O. JAMES.

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